SCIENTIFIC MEMOIRS

BY

MEDICAL OFFICERS OF THE ARMY OF INDIA.

EDITED BY

SIR BENJAMIN SIMPSON, M.D., K.C.I.E.,

SURGEON-GENERAL WITH THE GOVERNMENT OF INDIA.

PART IV.

1889.

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Are Choleraic Comma-Bacilli, even granting that they are the proximate cause of choleraic symptoms, really efficient in determining the epidemic diffusion of cholera?

BY

SURGEON-MAJOR D. D. CUNNINGHAM, M.B.,

BENGAL MEDICAL SERVICE.

In the present paper I propose, in the first place, to detail the results of a series of experiments on the behaviour of Choleraic Comma-Bacilli when introduced into water and soil, and subsequently to consider how far they are capable of affording an answer to the question contained in the title.

A.—Experiments on the behaviour of Choleraic Comma-Bacilli in water.

EXPERIMENT I.—A litre of water was taken from a tank of fair relative quality, situated in the compound of the General Hospital. The water was introduced into a clean glass beaker, and 0·5 C. C. of sterilised salt solution full of Comma-Bacilli from a pure tube-cultivation of twenty-four hours' growth added to it. The beaker was then covered with an inverted glass capsule and set aside on a table in the laboratory, where it was exposed to ordinary air-temperature.

Previous to the addition of the Bacilli to the water an Agar-Agar plate-cultivation had been inoculated with 0·5 C. C. of it. Two days later the cultivation contained one isolated patch of fungal hyphae, and between forty and fifty schizomycete colonies, many of them of very considerable size. Preparations were mounted from sixteen of the colonies—from all which appeared in any way to present distinctive features. On examination twelve different forms of Micrococci and Bacilli were recognisable. Not a trace of commas was to be found.

Two days subsequent to inoculation the water, which had originally been faintly turbid and yellowish, had deposited a thin greenish white sediment and become quite colourless and transparent. A plate-cultivation, inoculated with 0·5 C. C., was now set. On the subsequent day a very large number of schizomycete colonies had made its appearance, the great majority being of very

minute size and of the faint bluish tinge characteristic of colonies of commas in Agar-Agar plate-cultivations, but twenty-six large, prominent white ones, resembling those present in the cultivation from the water previous to contamination, being also present. On examination the small colonies were found to be composed of large, well developed commas of considerably greater size than those which were present in the material primarily used in inoculating the water. The large white colonies, on the other hand, consisted of short, thick straight Bacilli. A tube, which was inoculated from one of the small colonies, subsequently yielded a characteristic growth of commas, smaller than those in the plate-cultivation, but still larger than those in the primary tube.

Two days after the last plate-cultivation had been set another one was inoculated with a like amount of the water. On the following day it was found to contain a scanty crop of colonies. They were all of small size, and the numbers of bluish and white ones present were approximately equal. On the following day the number of colonies still remained very limited. Nine preparations from bluish colonies, and three from white ones, failed to show the presence of any recognisable commas.

Six days later another plate-cultivation of the water was set, as before. On the following day one or two very minute colonies, and two thin, widely diffused yellowish patches had made their appearance. Twenty-four hours later the entire surface of the plate was almost covered by a continuous thin yellowish stratum and gave off a very sour smell. A very few defined white colonies were also present. Six preparations were mounted. On examination these were all found to contain straight Bacilli, those corresponding to the diffused growth being distinguished by their slenderness.

Another similar plate-cultivation was set forty-eight hours after the previous one. Two days later it contained only one or two colonies, consisting of large straight Bacilli and very minute Micrococci.

Two days later, or in other words thirteen days after the water had been inoculated, another plate cultivation was set and the water was then thrown out. Seventy-two hours after, it only showed five small, white, circular colonies, all of them consisting either of short, straight Bacilli, or Micrococci.

EXPERIMENT II.—A fresh litre of water from the same source was inoculated with 1 C. C. of fluid from a tube-cultivation of commas of 72 hours' growth, which had been stirred up with a little neutral salt solution. The number of commas added was so great as to give rise to a faint, opalescent cloudiness in the water until diffusion had taken place.

Three days later a plate-cultivation inoculated with 0·5 C. C. of the water was set. On the following day a few colonies were visible on the plate. No commas could however be found. On the next day a limited number of bluish and yellowish colonies were present. Twelve preparations were mounted, and of these one was found to consist of large vacuolated commas.

Three days later the cultivation was again examined, as a considerable increase in the number and size of the colonies had evidently occurred. It had a peculiar disagreeable, musty, but non-choleraic odour. Six preparations were mounted and of these one contained small commas.

Five days after the beginning of the experiment a second plate was set as usual. Forty-eight hours later four dense, small white colonies had appeared. Three preparations were mounted and found to consist of large micrococci and short thick rods with rounded extremities.

Eight days after the beginning of the experiment a fresh plate was inoculated. Forty-eight hours later a very limited number of colonies had appeared, and the cultivation had a faint disagreeable but non-choleraic smell. Preparations were mounted from twelve colonies. On examination they were found to contain various forms of straight Bacilli, but nothing in any way suggestive of commas.

EXPERIMENT III.—Such having been the results in the case of a comparatively pure water, it appeared next to be desirable to experiment with one of bad quality. A supply of water was therefore procured from a foul tank liable to contamination by drainage. A litre of this was, as before, introduced into a beaker and 0·5 C.C. used to inoculate a plate-cultivation. Twenty-four hours later this was everywhere absolutely crowded with countless yellowish and pale bluish colonies, many of which were of very considerable size. Twelve preparations were mounted from those colonies which presented any features resembling colonies of commas, but on examination they were found all to consist of various forms of short, thick, straight Bacilli and Micrococci. This cultivation had an offensive but non-choleraic odour.

As this cultivation clearly showed that the water was excessively rich in schizomycetes and that 0·5 C.C. was much too large an amount to be used in inoculation for satisfactory plate cultures, another one was set which had been inoculated merely by means of twice dipping a needle first in the water and then in the fluid gelatine. On the following day this was found to be almost entirely covered by thin, diffuse, blue and yellowish films. At one place the yellowish coating had a decidedly greenish tinge. A few concrete bluish and white colonies were likewise present. The smell was sickly and disagreeable but non-choleraic. Six preparations were mounted and showed several forms of short straight Bacilli and Micrococci, but no sign of commas.

The water was now inoculated with 0·5 C.C. neutral salt solutions full of commas from a cultivation of 48 hours' growth, and two days later a plate was set, the gelatine having received the fluid transferred from the water by means of two dips of a needle. On the following day a limited number of concrete colonies of whitish and bright bluish tint had appeared, together with diffuse areas of yellow and bluish colour. The smell was sickly and suggestive of that of the flowers of *Bassia latifolia*, but not of a choleraic character. Six prepara-

tions were mounted from colonies, the appearance of which was most suggestive and one of them was found to consist of commas of large size.

Another plate was inoculated four days after the introduction of the commas into the water, and on this occasion only one dip of the needle was employed. Two days later the cultivation contained a very limited number of concrete colonies of bluish and yellowish tint.[1] Twelve preparations were mounted, but in none of them was there anything even suggestive of commas.

Six days after contamination of the water another plate was set, this time with 0·5 C.C. On the following day the cultivation showed only a very few concrete colonies and two diffused patches. It was quite devoid of any perceptible smell. Eleven preparations were mounted but in none of them were commas present.

EXPERIMENT IV.—A litre and 100 C.C. of water from the same foul tank were introduced into a clean, plugged flask and boiled strongly for periods of half an hour on three successive days. Twenty-four hours later a plate was inoculated with 1 C.C. of the water. On the following day only three or four small colonies were present, and, as these all consisted of a merismopædic form of Micrococcus which at that time was excessively abundant in the air of the laboratory, it appeared probable that the growth was due to local atmospheric contamination, and not to the persistent vitality of any of the schizomycetes originally present in the water. The latter was now inoculated with 1 C.C. of salt solution, crowded with commas from a tube-cultivation of seventy-two hours' growth. The flask was well shaken up, and after an interval of an hour 1. C.C. of water was used to inoculate a plate-cultivation. On the following day the plate showed an even diffusion of innumerable small bluish colonies. Three preparations were mounted, and were all found to consist of pure cultivations of characteristic commas. Two days later the plate was again examined. The colonies of commas had increased greatly in size, and the cultivation had a strong characteristic choleraic smell.

Three days after the inoculation of the water a second plate-cultivation, containing 1 C.C. of water, was set. On the following day this had a characteristic choleraic odour and was absolutely crowded with small colonies of typical commas. The cultivation was almost a pure one, only one or two large concrete, yellowish colonies, composed of other schizomycetes being present.

Five days after the beginning of the experiment another plate was inoculated by the fluid transferred by one dip of a needle, as the amount of commas indicated by the previous experiment was still so considerable as to be likely to lead to overcrowding of the gelatine if large quantities of water were used in inoculation. Only three or four colonies had developed in this cultivation after twenty-four hours, and only one of these presented any features suggestive of a colony of commas. A preparation was mounted from this, and a second from one of the other colonies. In neither were commas present.

[1] The cultivation was almost odourless.

Another plate-cultivation, containing 1 C. C., was set six days after the commencement of the experiment. After a period of twenty-four hours it was found to be crowded with colonies. The majority of them were prominent and yellowish, and clearly not composed of commas. A few blue ones were, however, also present. The smell of the cultivation was faint and of a somewhat spirituous character. Two preparations were mounted, one from one of the yellow colonies and the other from a blue one. The former contained short oval rods, the latter comma-bacilli.

Another cultivation, again containing 1. C. C. of the water, was set two days later. After forty-eight hours it contained an abundance of prominent ochre-tinted colonies and a few small flattened bluish ones. It was devoid of smell. Six preparations were mounted. Those from yellow colonies consisted of short oval rods and micrococcoid bodies, those from blue ones contained short, thick, straight rods. No commas were present in any of them.

Eighteen days after the beginning of the experiment another cultivation, containing 1. C. C. of water, was started. Two days later it contained a considerable number of large colonies of various kinds. The smell was very faint and slightly acid. Six preparations were mounted, and one of these was found to contain small commas.

Seven days later—25 from the beginning—a similar preparation was set. In the course of 48 hours a considerable number of bluish and yellowish colonies had made their appearance. Six preparations were taken from bluish colonies, as the others were clearly not composed of commas. All of them were composed of long filaments, built up of straight rods.

Thirty-one days from the beginning of the experiment another cultivation was set as before. A limited number of colonies made their appearance in it. Some of these were large, prominent, and yellowish; others were smaller and of a pale bluish tint. The yellowish ones consisted of diplococci, the bluish ones of filaments similar to those present in the previous cultivation.

Finally, forty-seven days after the inoculation of the water, another cultivation was started, containing as before 1 C. C. of the water. Hardly any colonies appeared in it. It had a faint but non-choleraic odour, and no commas could be detected in it.

EXPERIMENT V.—Another litre of water from the same polluted tank was placed as in the previous experiments and 1 C. C. of salt solution full of commas from a cultivation of ninety-six hours' growth added to it. Nine days later a plate-cultivation, containing 1 C. C. of the water, was set. It became covered by an almost continuous stratum of a bright yellow colour, and the smell was faint and non-choleraic. Preparations taken from it contained micrococci and several forms of straight Bacilli, but no commas were to be found. Nineteen days after the commencement of the experiment another similar cultivation was started. It produced numerous concrete colonies and a large diffused gelatinous patch of

greenish-yellow colour. The smell was very faint and non-choleraic. No commas could be found in any of the preparations taken from it.

In this experiment, as in the others in which the water was not boiled, a development of green algae made its appearance at the bottom of the beaker after some time, and active evolution of oxygen then set in. The phenomena afforded an excellent demonstration of how a body of water originally very foul will gradually purify itself if only the addition of new supplies of filth be prevented.

B.—Experiments on the behaviour of Choleraic Comma-Bacilli in Soil.

EXPERIMENT I.—One hundred grammes of freshly dug garden earth were set in a glass beaker and 50 C. C. of fluid and flocculi from a choleraic evacuation forty-eight hours old, poured over it. The presence of large numbers of commas in the stool had been previously determined. The beaker was now allowed to stand uncovered beneath a bell glass until the fluid had all soaked down into the earth with the upper stratum of which it had been mixed. It was then covered with a watch glass and set aside beneath a bell glass.

Twenty-six days later it was examined. The earth was still moist and emitted a strong, earthy smell. A little was removed from the surface, stirred up in a watch glass with sterilised salt solution, and a plate-cultivation inoculated with the fluid conveyed by one dip of a needle. On the following day it showed a few large, white colonies resembling those of the Hay-Bacillus. Twenty-four hours later they had increased in size and were now accompanied by a few bluish ones of considerable size and by an abundance of very minute, yellowish-white ones. Two colonies of the merismopædic micrococcus, previously alluded to as very prevalent in the air of the Laboratory for some time, were also present. The cultivation had the peculiar, strong, bat-like odour which had been noted in similar cases previously. Six preparations were mounted. Four of them consisted of small, straight Bacilli, one of Hay-Bacillus, and one of the merismopædic micrococcus.

Four days later another similar cultivation was set. After forty-eight hours it showed abundant growth. The general surface of the gelatine was of a pale ochre-tint, due to a thin diffused growth, while a few large white concrete colonies, and one or two pale bluish ones, were also present. It had the same peculiar smell as the previous cultivation, but not so strongly developed as in it. No commas were to be found.

EXPERIMENT II.—The growth of commas in four pure tube-cultivations was mixed with 50 C. C. of distilled water and poured over 100 grammes of garden earth in a beaker. A fortnight later the earth was still moist. It had a faint, earthy smell, and the surface was coated by a thin gelatinous layer, consisting of fragments of agar-agar, and showing numerous small whitish patches apparently

due to the presence of schizomycete colonies. A plate-cultivation was inoculated as in the previous experiment. On the following day it contained an enormous number of colonies and emitted a strong, offensive, but non-choleraic odour. Twenty-four hours later the smell had become faint and choleraic. The surface of the gelatine presented a generally diffused greenish-yellow colour, due to a thin overgrowth, consisting of long, slender Bacilli. Numerous defined colonies of small straight Bacilli were also present, but no commas could be detected.

Sixteen days after the beginning of the experiment a new cultivation was set Forty-eight hours later the surface of the gelatine was of a generally diffused, brilliant greenish-yellow colour. It was almost odourless and thickly bestrewn with large flattened patches, which were greenish-blue and greenish-yellow by transmitted light, and there were also generally diffused brilliant yellow haloes around them, and in many places over the general surface. In addition, there were very many small, discrete, yellowish colonies embedded in the substance of the gelatine. Small straight Bacilli abounded, but no recognisable commas were to be found.

EXPERIMENT III.—One hundred grammes of dry pulverised garden-earth were put into a glass beaker and 50 C. C. of sterilised salt solution containing the commas of two tube-cultivations of seventy-two hours' standing poured over it. The beaker was then covered and set aside beneath a bell-glass.

Three days later a plate-cultivation was inoculated by means of a needle which had been immersed in salt solution, in which a little of the earth had been stirred up. On the following day the surface of the gelatine showed an almost universal yellowish coating, and numerous concrete colonies. The generally diffused coating was due to a large straight Bacillus, and some of the concrete colonies consisted of commas. On the following day the cultivation had a very peculiar smell, of mingled choleraic and sour character. A second plate cultivation was now—five days after the beginning of the experiment—started as before. On the following day it contained numerous large colonies, but the smell was not choleraic. Large, straight Bacilli abounded, together with long filaments, consisting of slender straight ones, and very short, thick oval forms. No commas could be detected. On the following day, however, one preparation was obtained, containing slender commas, isolated and in chains. Ten days after the commencement of the experiment another plate-cultivation was set as before. Two days later the surface of the gelatine was to a great extent covered by a continuous growth of a yellowish tinge. A certain number of isolated colonies were also present, some of them resembling the continuous stratum in appearance. Twelve preparations were mounted and were all found to consist of large, thick, straight Bacilli.

As the surface portions of the soil now appeared to be devoid of commas, they were removed, and a plate was inoculated from earth near the bottom of the beaker. This was done thirteen days from the commencement of the experiment. On the following day the cultivation was to a great extent covered by continuous,

thick, yellowish strata, with a few more prominent masses. It had no perceptible smell. No commas could be found, the growth apparently consisting entirely of large, straight Bacilli

EXPERIMENT IV.—One hundred grammes of dry garden-earth were triturated with about thirty grammes of fresh fæcal matter and the mixture set as usual in a covered beaker. The fæcal matter was neutral in re-action. Forty-eight hours later the earth appeared to be everywhere penetrated by white fungal hyphæ, and on the surface a crop of young sporangia, of a species of mucor, had begun to appear. Fifty cubic centimetres of neutral salt solution full of the commas from two tube-cultivations of forty-eight hours' growth were now poured over it and the beaker again set aside. Two days later a stratum of brownish alkaline fluid, with a distinct thin scum, was still present on the surface. Some of this, and some of the surface earth, were mixed with neutral salt solution and a plate-cultivation of it set as usual. On the following day this had a sickly, somewhat choleraic smell. The surface was covered by yellowish and bluish colonies, and full of small interstitial ones. Six preparations were mounted, and in two of them commas were present.

Six days after the addition of the commas to the earth another plate-cultivation was started. Forty-eight hours later it was crowded with concrete yellowish colonies, and also contained some large, diffused patches of yellowish and bluish colour. The smell was non-choleraic and like that of the flowers of *Bassia latifolia*. Twelve preparations were mounted, but none of them contained commas.

Eight days after the commencement of the experiment another plate-cultivation was inoculated from the earth. On the following day it had a faint non-choleraic smell and was crowded with concrete colonies. Some diffuse patches, of yellowish and bluish colour, were also present. Twelve preparations were mounted from colonies which in any way resembled colonies of commas, but none of them contained commas. Two days afterwards the cultivation had a strong smell of bats. Another set of preparations was mounted, but, as before, contained no commas.

EXPERIMENT V.—Twenty-five grammes of fresh fæculence was triturated with seventy-four grammes of dry garden-earth. Water was then added and the mixture thoroughly stirred up and boiled over a Bunsen flame until nearly dry. It was then transferred to a clean glass beaker and the latter set in a boiling water-bath for half an hour. On the next day the beaker was kept in the bath for an hour, and the same process was repeated on the two following ones. At this stage of the experiment a plate-cultivation was inoculated from the soil by the usual method. On the following day it was found to be full of yellowish defined, circular colonies. The smell was faint and non-choleraic. Preparations showed that the colonies were apparently all composed of a short, thick, straight Bacillus. Twenty-four hours after the previous heating the beaker

was again heated in the bath, and finally for several hours over a Bunsen flame On the following day the growth in two tube-cultivations of commas was mixed. with some salt solution and poured over the earth.

Two days later a plate-cultivation was set as usual. On the following day it contained immense numbers of colonies, large yellowish ones and minute blue ones being apparently present in approximately equal numbers. The yellowish colonies consisted of the form of Bacillus which appeared in the cultivation of the earth previous to the additions of the commas, the blue ones of commas. These were of rather large size and somewhat pale and granular. The smell of the cultivation was bat-like.

Four days after the introduction of the commas another plate-cultivation was set. On the next day it presented microscopic characters similar to those present in the previous cultivation, the small blue colonies, however, being relatively more abundant. The smell was a curious mixed one, the bat-like odour being accompanied by a characteristic choleraic one. The results of microscopic examination were identical with those in the previous case, the large yellowish colonies being composed of large, straight Bacilli and the blue ones of commas. On the following day the cultivation had a very strong, unmixed odour of bats.

Seven days from the beginning of the experiment another cultivation was started. Seventy-two hours later it had a strong, decidedly choleraic smell, and was crowded with colonies. These, as before, consisted of large yellowish and of small blue ones. The blue ones consisted of commas, both isolated and in the vibrionic condition.

Ten days after the addition of the commas to the earth another plate was set. It was examined after forty-eight hours and found to have a faint smell, and to be full of colonies similar to those in the previous cultivation. As in it, the blue colonies contained commas of slender form and only staining feebly.

On the twelfth day another cultivation was set. Twenty-four hours later it was crowded with minute colonies and had a very faint, rather pleasant, vinous smell. On the next day the smell remained unchanged; no commas could be found, the colonies seeming to consist of two forms of straight Bacilli only, one presenting short, thick, isolated rods, the other slender ones, both separated and associated in filaments.

On the fourteenth day another cultivation was set. Twenty-four hours after it was absolutely crowded with colonies, the majority of which were of large size and yellowish colour. The smell was disagreeable and bat-like. Small slender commas were present in half the preparations, which were mounted.

Another cultivation was set on the fifteenth day. Two days later it had a faint, choleraic smell, and was covered with bluish and yellowish colonies, of various sizes. Five, out of six, preparations which were mounted contained commas.

On the twenty-first day another plate was inoculated. Two days later it had a strong disagreeable smell, and was covered with large blue and yellow colonies. Three out of four preparations contained commas.

Twenty-eight days after the inoculation of the soil another plate was set. Forty-eight hours later it had a strong but somewhat choleraic smell, and contained numerous colonies, the majority of a yellowish tint, but a few bluish ones also being present. The former consisted of large, thick, straight rods, the latter of small commas.

On the forty-seventh day a final cultivation was set. In the course of twenty-four hours it had become full of colonies, like those in the previous case, and had acquired a faint, somewhat choleraic smell. The blue colonies, as before, consisted of commas.

EXPERIMENT VI.—Some fresh fæcal matter was triturated with 100 grammes of dry garden-earth and set in a covered beaker as in the previous experiment. Two days afterwards the growth of two tube-cultivations of commas of four days' date was stirred up in sterilised salt solution and poured over the earth. Nine days later the soil was still very moist, with a slightly fæculent odour and a neutral re-action. A plate-cultivation of it was set. Numerous schizomycete colonies and patches of fungal hyphæ appeared in it. It had no perceptible odour and no commas could be found in it.

On the nineteenth day the surface of the soil was still quite moist, emitted a faint stercoraceous odour, and was covered by a puffed-up sheet of fungal mycelium, with radiant, whitish tufts of hyphæ. A plate-cultivation was set. On the following day it was covered by a continuous stratum, of ochre colour, which involved isolated colonies of several kinds. The smell was faint and disagreeable but non-choleraic. No commas were to be found in it.

Taking the experiments as a whole, they indicate that comma-Bacilli, when introduced into soil and water of very different qualities, so long as these retain their natural condition, tend to disappear very rapidly.

The periods of survival are shown in the following tables :—

Table I.—Periods of Survival of Commas in Water.

No. of Experiment.	Quality of Water, &c.	Period.
No. I.	Fairly clean : unboiled .	Disappearance in 4 days.
,, II.	,, ,, .	,, ,, 5 ,,
,, III.	Foul : unboiled . .	,, ,, 4 ,,
,, IV.	,, boiled . .	,, ,, 25 ,,
,, V.	,, unboiled . .	,, ,, 9 ,,

Table II.—Periods of Survival of Commas in Soil.

No. of Experiment.	Quality of Soil.	Period.
No. I.	Garden earth . .	Absent after 26 days.
„ II.	„ „ . .	„ „ 14 „
„ III.	„ „ . .	„ „ 10 „
„ IV.	Garden earth and fæces .	„ „ 6 „
„ V.	Garden earth and fæces; boiled.	Still present after 47 days.
„ VI.	Garden soil and fæces; unboiled.	Absent after 9 days.

The period during which they retain their vitality is very much shorter than that in cases of pure cultivations, for in Calcutta successful cultivations may be obtained from tubes up to a period exceeding sixty days. The fact of their rapid disappearance from foul water if left in its natural state has been already clearly demonstrated by Koch's celebrated observation on their occurrence in the water of a tank in Calcutta during a period of epidemic prevalence of cholera in the surrounding huts, *i.e.* during a period when fresh supplies of them were liable constantly to gain access to the water—and their disappearance shortly after the cessation of supply consequent on the termination of the outbreak.

Two exceptional cases are present, one in the set of experiments on water, the other in that on the soil, but in both these the materials had not been left under normal conditions previous to the access of the commas, but had been exposed to the influence of temperatures high enough in the case of the water to practically sterilise it, and in that of the soil to determine the destruction of the filamentous fungi, and probably of large numbers of the schizomycete organisms originally present in it. In both cases commas persisted longer than in the other experiments, and in that of the soil the persistence was such as to render the result practically identical with that in an ordinary pure tube-cultivation. The shorter period of persistence in the case of the boiled water may be ascribed with probability to the much smaller amount and, therefore, more rapid expenditure of nutritive material present. The proportion of nutritive material present in a medium seems not only to determine the duration of vitality in the commas, but also induces differences in the development of the individual specimens. This is, of course, well known in connection with the appearance of "involution" forms in nearly exhausted media, but, in addition to this, variations in size without alteration in formend to occur in many cases. There is frequently

a tendency to an increase in size of the individual commas connected with transference to a less nutritive medium, the conditions appearing in one case to make for rapid multiplication, in the other for continued growth, of individuals.

There can be little doubt that the persistence of the commas in the case of the heated media must be regarded as due to the artificial advantage which they there acquired in the struggle for existence. The temperature was not sufficient to produce any important change in the water or soil, save reducing the number of living organisms in them. In regard to the mixture of soil and fæculent matter, it is easy to realise so far exactly why such a reduction should have been of advantage to the commas. The change which primarily takes place in connection with decomposing fæculence of normal quality in Calcutta is an excessive development of filamentous fungi, which is accompanied by extreme acidity and a temporary suppression of schizomycete evolution generally, and, from what we know of the nature of the comma-bacilli, such conditions must be especially unfavourable to them. In the case of the water, however, there is no such development of filamentous fungi present, so that the boiling here must have acted in some other fashion. It seems probable that here also it was the partial sterilisation that was the efficient factor, due to its destructive effects on the schizomycetes normally present. The removal of these was calculated to favour the commas in the struggle for existence, certainly by increasing the amount of food at their disposal, and also, quite possibly, by arresting the formation of waste products, arising in connection with the development of other forms and directly toxic in their effects on the commas. In the case of the soil the effect would not be limited to the suppression of the higher fungi, but would also partially extend to the schizomycetes originally present in it and the fæculence with which it was mingled; and, although actual sterilisation was not attained, must have destroyed large numbers of them and may have determined the absence of species specially antagonistic to the commas.

In the discussion of the vexed question of the relation of Comma-Bâcilli to cholera generally, it would appear to have been very commonly assumed that, in order to a definite decision of it, all that was necessary was to show that choleraic symptoms accompanied the multiplication of commas within the intestinal tract, or the introduction of special toxic matters of the nature of ptomaines formed by them into the system, by that or other paths. Great diligence has therefore been applied to experimental research in this direction, and the fact that morbid effects are frequently the result has, by those who regard these as of truly choleraic character, been regarded as a final one, while another school of observers has devoted all its energies to endeavour to show that the symptoms are not really choleraic in their nature. It appears to have been, to a great extent at all events, lost sight of that there are really two subordinate questions involved in the major one of the relation of the Bacillus to the disease. These are first:—Are commas or their products capable of giving

rise to choleraic symptoms? and second:—Are commas alone efficient agents in determining the epidemic diffusion of the disease? That they really are quite distinct ones is, however, evident even to casual consideration, and it is further clear that the latter question is the one of really practical import. Had it been unequivocally and finally shown that, as the result of laboratory experiments, choleraic symptoms were induced under the influence of the action of Comma-Bacilli or their products on the animal organism, a great advance in our knowledge of the disease would have been made. There could, under such circumstances, be no more discussion of the question whether the presence of commas were causative of, or consequential to, the presence of the choleraic state, but this alone would, so far, be merely a scientific gain. Did cases of choleraic poisoning only occur in the same isolated fashion as those of poisoning by the higher fungi do, their practical importance would be inappreciable. It is of scientific interest to us to know that certain species of the higher fungi are poisonous, and it would be of scientific interest to us to know that Comma-Bacilli were so also, and that they produced certain definite effects on the animal organism. But the great practical interest in regard to cholera depends entirely on the fact that cases do not only occur isolatedly but in epidemic fashion, so that, even had it been definitely established that the symptoms of the disease were due to the presence of commas, very little practical gain would have been attained.

Assuming, as a matter of hypothesis, that those observers are in the right who maintain that Comma-Bacilli do give rise to choleraic symptoms, let us now endeavour to ascertain what evidence there is in regard to the part which they play in determining epidemic diffusion of the disease. The experiments on the influence of the Bacilli on the animal organism are separable into two classes,—1st, those in which choleraic symptoms are said to be developed only under the influence of certain adjuvant measures; and, 2nd, those in which they are stated to occur independently, and simply as the result of the access of the Bacilli to the organism. Koch's experiments may be taken as an example of those of the first class, and Hueppe's experiments with "Arthrospores," of those of the second. According to Koch the introduction of commas into the digestive canal of animals is, under normal conditions, followed by no result, because from their extreme susceptibility to the action of acids they are killed by the gastric juice, and do not therefore reach their normal site of development in the small intestine in a state to do any mischief. Adjuvant means have, therefore, to be employed, consisting in establishing temporary alkalinity in the stomach and injecting opium and alcohol into the peritoneal cavity, and only when this has been done is the access of the parasite to the digestive track by the normal path followed by morbid effects. From this it would appear that in the presence of normal healthy conditions on the part of the recipient organisms the Comma-Bacilli are incapable of doing any harm, and that it is only with the concurrent presence

of abnormal ones—of conditions arising in the case of the experiments as the result of special preparatory measures, and in that of the disease when originating naturally as the result of a dyspeptic condition—that they are able to do so. Cases of cholera must then depend on the presence of such a condition antecedent to the access of the Bacilli. But, if this be so, it is clear that, in order to the occurrence of epidemic diffusion of the disease, we must have not only diffusion of the Bacilli but coincident epidemic diffusion of the necessary dyspeptic condition. The mere diffusion of the Bacilli in however great amount would be incapable of determining the epidemic occurrence of the disease. They must, therefore, necessarily be regarded as only playing a secondary part, and the presence of the dyspeptic condition, as the really important factor. In every case of epidemic diffusion, according to this theory, we must assume the coincident spread of two factors, of the Bacilli and of the conditions allowing of their producing any effect, and the latter must be the really important one, for, if the Bacilli are incapable of producing any effect, it is of no matter how freely they are diffused. This is, of course, quite in accordance with a great mass of evidence which has been brought forward by various observers, and very specially by Von Pettenkofer, showing that development of epidemics in localities does not necessarily run parallel with the facilities for the introduction of materials from previously infected ones, but the theory ceases to be a contagionistic one and really becomes a localistic one. If pushed to its logical conclusion it must come to regard the local conditions determining the presence of the necessary antecedent state in the recipient organisms as the really important factor, and to conclude that in order to secure the absence of epidemics it is the development of these conditions, and not mere access of the Bacilli, which must be prevented.

In proceeding to consider the bearing of the second class of experiments—those in which choleraic symptoms are said to follow on mere access of the Bacilli to the organism of animals without the presence of any special adjuvant conditions—on the question of the relation of the commas to epidemic diffusion, it may appear at first sight as though a different conclusion must necessarily be arrived at, as though the evidence showed that the commas really were efficient factors for the determination of epidemic diffusion. Had it been shown unequivocally that the Bacilli in their ordinary normal condition were capable of inducing the development of cholera in healthy organisms, this would no doubt to a certain extent have been the case, but this has never been affirmed hy Hueppe. What he maintains that he has experimentally proved is, that under certain conditions of defective nourishment and of exposure to special temperatures the Bacilli no longer follow their common course of development, but give origin to peculiar small, round, highly refractive cells which he regards as Arthrospores, and describes as endowed with much greater powers of resistance to the influence of various external conditions than that which is

possessed by the commas in their normal state.[1] For example, while the ordinary commas are killed by exposure to drying for a few hours, the Arthrospores are said to retain their vitality under similar circumstances for weeks, and while commas are incapable of surviving the action of the normal gastric juice the Arthrospores can survive it, and are therefore capable, without any assistance, of inducing cholera in healthy animals to whose digestive canal they have gained access.

The formation of Arthrospores, according to this, comes to replace the adjuvant measures or the naturally developed dyspeptic conditions of the previous class of experiments. In order, however, to show that the Bacilli alone are efficient determinants of epidemic diffusion of cholera, it would still be necessary to show that Arthrospores are normally formed under the ordinary conditions to which the parent commas and spirochætal forms are exposed. Assuming that Hueppe is perfectly correct in regard to the formation, nature, and properties of the Arthrospores, there is as yet an absence of evidence to show that they are formed under normal conditions. Their development has only been observed under the influence of special conditions, and one of these apparently is that the parent Bacilli were in pure cultivations.

There is a great tendency to assume that all phenomena presenting themselves in pure cultivations are to be taken as sufficient indices to the normal behaviour of the special organism under observation in other circumstances. But this, of course, by no means necessarily is the case, and there is no evidence to show that in the case of the Comma-Bacilli any development of Arthrospores would occur, even under the influence of exhaustion of medium and special temperature, if the cultivations were no longer pure ones; or, in other words, were the Bacilli exposed to a struggle for existence. On the contrary, there is evidence indicating that under such circumstances there is no tendency to the development of Arthrospores, but merely to rapid and final disappearance of the Bacilli. Taking the case of the present experiments on their behaviour in soil and water of various degrees of pollution—that is, in media resembling those to which they must normally tend to gain access in greatest numbers—the evidence was entirely in favour of rapid extinction and against the development of any specially resistent forms. These results are not isolated ones, for, although it has been affirmed that Comma-Bacilli are specially inimical to certain saprophytic Schizomycetes,[2] the general consensus of evidence and opinion must lead one to regard this as a very exceptional phenomenon, and to believe that, generally speaking, and under normal circumstances, they are singularly incapable of holding their own in the struggle for existence. When Comma-Bacilli are imported into any locality they do

1 Ueber die Dauerform der sogenannten Komma-bacillen—F. Hueppe. Deutschen Medicinischen Wochenschrift, 1885.

2 Bakteriolog. Untersuchung von choleraverdächtigen Fällen unter erschwerenden Umständen. Max Gruber, 1887.

not find that it has been specially sterilised for them, it is already fully occupied by native Schizomycetes with which a struggle must be maintained, and under normal circumstances, even in localities such as Calcutta, the experimental evidence goes to show that they very rapidly succumb to it.

But this Laboratory evidence is quite in accordance with that which presents itself on a large scale in connection with the phenomena of the occurrence of epidemics of cholera. As we have already seen, Koch has recorded a striking example of the rapidity with which Comma-Bacilli will disappear from large bodies of foul water to which they have gained access, and, if we are to regard the commas as in any way causally related to cholera, the proportion of cases in which they die out rapidly in localities to which they have gained access must be very great.

Under normal circumstances the local conditions, then, would appear to be so unfavourable to their multiplication and persistence that, however efficient they may be as producers of choleraic symptoms, they can neither persist or multiply sufficiently to give rise to any appreciable effects. The normally unfavourable conditions in any locality must to a great extent be of two classes —1st, conditions tending to diminish the amount of suitable nutritive materials for the Bacilli in the media to which they gain access; and, 2nd, conditions tending to increase the intensity of the struggle for existence to which they are exposed. The actual intensity of the struggle must, of course, depend in part on the numbers and in part on the nature of the organisms present in the media at the time of access. In regard to India, at all events, there can be little doubt that it is the latter class of conditions which, except in certain desert areas, must be the influential ones, the general quality of soil and water in most places certainly not being such as to render it likely that the repressive agent is a want of nutritive material. It is of course, however, possible that elsewhere, in localities with specially pure water and soil, want of nutritive material may really play an important part in this respect.

Be this as it may, what the experimental evidence goes to prove is that under normal circumstances local conditions of soil and water are such as to be very inimical to the persistence or multiplication of Comma-Bacilli, and that, therefore, under normal conditions of locality, the latter are not likely to be able to establish themselves to such an extent as to give rise to any appreciable effects in the production of cases of cholera. In order, then, to the occurrence of cholera in epidemic fashion in any ordinary locality, and assuming the Bacilli to be really causally related to the disease, it is clear that we must have alterations in the local conditions. Such alterations are necessary according to Koch's theory in order to permit of the presence of commas in sufficient numbers, and also to give rise to the development of abnormal dyspeptic conditions in the inhabitants, of a nature to render them suitable recipients for them. Here the commas themselves clearly are reduced to playing a very subordinate

rôle indeed in so far as epidemics are concerned. If Hueppe's observations be accepted the subordination would, of course, not be so great, but it would still be present, as it would still be necessary that the local conditions should be adapted to allow of the multiplication of the Bacilli and of the development of Arthrospores from them. In both cases alike it would be the presence of abnormal local conditions, and not mere importation of commas, that would be the primary determinant of epidemic occurrence of the disease, and the prevention of the development of such local conditions would be the end to be aimed at in the endeavour to prevent such an occurrence.

As we have already seen, the deviations from the normal tending to favour the establishment of the Bacilli must probably lie either in increase of suitable nutritive supply, or in the lowering of the struggle for existence with other organisms, and the practical measures to avoid the establishment must therefore be aimed either at diminution of supply of suitable nutritive material or the increase of the struggle for existence. The former aim will certainly be more or less attained by improvements in local sanitation tending to increased purity of soil and water, the latter would no doubt be to some extent achieved by the same method, for diminished nutritive supply in itself implies increased struggle but could only be satisfactorily arrived at by means of an accurate acquaintance with the nature of the organisms most inimical to the Bacilli. Even could such information be attained there can be no doubt that the endeavour to diminish nutritive supply would be the preferable course to follow, as it by no means would necessarily follow that the organisms which were inimical to Comma-Bacilli should be innocent or desirable inhabitants of a locality. As Naegel long ago pointed out,[1] it is quite possible that excessive dirt in a locality may be an efficient cause for the prevention of the prevalence of certain forms of disease in it, the excess of saprophytic organisms tending to the suppression of more or less parasitic ones, but no one could regard it as, therefore, desirable to increase the accumulation of dirt.

It is worth while to consider the principal ways in which the struggle for existence to which the Comma-Bacilli are exposed in any locality to which they gain access might be diminished. It might be diminished simply as a result of diminution in the number of conflicting organisms generally, the nutritive supply remaining at the same time unaltered. The greater the diminution was the more would the locality come to resemble a sterilised medium in its relation to the Comma-Bacilli. Diminution might also be dependent, not on mere general decrease in the number of organisms present, but on special decrease or disappearance of organisms specially inimical to Comma-Bacilli, either directly or indirectly by means of their products. Thirdly, it might be dependent on the presence of organisms which, in place of being mere competitors for nutrition or directly inimical, were positively favourable to their development.

[1] Nägeli, C.v, Die niederen Pilze in ihren Beziehungen zu den Infectionskrankheiten und der Gesundheitspflege. München und Leipzig.—R. Oldenburg, 1887.

That such a relation may exist even between Bacilli is well known, and a very striking example of it has presented itself during the current year in the Laboratory in Calcutta. In it the relation has advanced so far as to come to be one of actual commensalism, or perhaps rather of parasitism, in so far as one of the associated species of Bacilli is concerned. The favouring or host-species was apparently merely the common Hay-Bacillus, and was readily susceptible of pure cultivation. The other was a much smaller, slender form in which the rods were either isolated or associated in filamentous fashion. The rods and filaments are in themselves colourless, but their presence in any cultivation of the Hay-Bacillus is soon revealed by the gradual development of a red tint passing on gradually into a splendid scarlet. When first observed the growth manifested itself spontaneously in connection with a cultivation of the Hay-Bacillus derived from a sample of blood which had been obtained from a supposed case of anthrax. Here, after the cultivation was some days old, and when the surface of the gelatine was covered by a thick, yellowish stratum of the Hay-Bacillus, a bright scarlet, shining patch somewhat elevated above the surrounding surface, and contrasting beautifully with it, made its appearance. This gradually spread, and ultimately the entire surface of the Hay-Bacillus became of the same scarlet hue. Very many attempts have been made to procure pure cultivations of the intrusive species, but always in vain. Plate-cultivations have yielded pure growths of the Hay-Bacillus, and so have tube-cultivations inoculated from others in which the mixed growth is at an early stage of development. The latter fact is due to the very different rates of growth of the two species when they are simultaneously introduced to a new medium. Even where the inoculating material is derived from tubes of long duration, and in which the elements of the Hay-Bacillus are only present in relatively very small numbers and in a condition of evident extreme involution, a yellowish halo of them in pure, or almost pure, condition almost invariably manifests itself around the scarlet point of the inoculation, and by inoculating from this area in one or two successive cases a stage is arrived at in which the yellow growth alone makes its appearance and retains the character of a pure cultivation permanently. The difference in the rate of growth of the two Bacilli is, to a considerable extent, due to the fact that the medium alone is necessary for the growth of the Hay-Bacillus, while that of the other species is dependent on the pre-existence of its companion. That this is so comes out very clearly in cases where a tube which has become occupied by a large growth of the Hay-Bacillus as the result of previous inoculation is inoculated with the material of a mixed growth of long duration in which the other species is present in excess; for in such cases red colouration very rapidly manifests itself over large areas, in place of spreading slowly over the surface in the track of its host. The relation between the two species would appear to be one rather of true parasitism than of commensalism, for there can be no doubt that the growth of the

Hay-Bacillus is checked just in proportion to the amount of the other species, which is inoculated along with it, and the older any cultivation is and the more the latter species comes to abound in it, the more do involution-forms of the Hay-Bacillus tend to abound. . Plate-cultivations, even from the oldest sources, in which the Hay-Bacillus is at a minimum, fail to yield pure cultivations of the other species, because when isolated from the former its elements do not find the conditions necessary for their further development.

An example of this kind illustrates, on the one hand, the favourable influence which the presence of one species of Schizomycete may exert on the development of another, and on the other hand the possibility of the determination of obstruction or suppression of development under a similar association. In this case the presence of the Hay-Bacillus determined the growth of the other species, and the growth of the latter obstructed the development of its host.

In the light of such facts, it becomes readily conceivable how variations in the local conditions in any area of a nature to be quite inappreciable to ordinary observation may determine the development or the extinction of organisms imported into it, and therefore, in cases where these are of pathogenic nature, the presence or absence of special forms of disease.

But variations in local conditions may tell in this direction in another fashion also; they may not only determine the possibility of the existence of pathogenic organisms in the area, they may also tell on their capacity for producing their specific effects on the organisms of the inhabitants. If we accept Koch's theory of the epidemic diffusion of cholera, the essential coincident epidemic diffusion of an abnormal dyspeptic condition may, quite conceivably, be due to the presence of abnormal organisms in the affected localities.

View the question as we may, however, the paramount importance of local conditions and the subordinate and secondary rôle which Comma-Bacilli must play in reference to epidemic diffusion of cholera is very evident. The more the facts are considered, the more evident does it become that no contagionist theory will account for the phenomena of epidemic diffusion of the disease, and that the only one which will do so must be of the nature of that which has been so long advocated by the observer who has done most to advance our knowledge of the subject. It is very possible to differ from Von Pettenkofer in regard to matters of detail, and yet to believe that he has all along been in the right in insisting on the primary importance of local conditions. It is of little moment whether these act by preventing or favouring the development of a germ, the multiplication of a mature form, the presence of specially resistent elements, or receptivity on the part of the inhabitants; in any case, they must be the primary determinants, and those, too, which may be hopefully contended with. A solitary living Bacillus, imported into a favourable locality, may, assuredly, if the Schizomycete origin of cholera be a fact, give rise to an epidemic, and on purely theoretical, as well as on practical grounds, the futility of measures aimed at pre-

venting the possibility of such importation needs no demonstration. But if it be allowed that epidemics can only arise under the co-operation of certain favouring local conditions, a hopeful field for efforts at prevention is at once opened out, and with the practical recommendation that the improvements in local sanitation which they necessarily imply will not have been absolutely fruitless, even should they not at once arrive at their special aim in reference to cholera. On every occasion on which quarantine or similar measures attempting to prevent importation are enforced, and cholera, in spite of them, succeeds in establishing itself, even the most ardent believers in the benefit of such procedures must allow that a futile waste of effort and money has taken place, but any improvement in local conditions must remain a benefit, even though its immediate purpose be not achieved.

CALCUTTA :
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Notes on the Life-history of *Ravenelia sessilis*, B. and *Ravenelia stictica*, B. and Br.

BY

D. D. CUNNINGHAM, M.B.,

BENGAL MEDICAL SERVICE.

During the past few years I have, as opportunities presented themselves, been accumulating notes on the life-history of the two species of Ravenelia mentioned in the heading, and as these now are fairly complete save in regard to one or two details, it appears to be worth while to publish them, more especially in consideration of the very imperfect knowledge which has as yet been arrived at in regard to the peculiarities of the genus. In so far as I am aware, all that is yet known is that Ravenelia is a genus of the Uredineæ producing uredospores and very peculiar teleutospores. In regard to the development of the latter, however, there would appear to be no accurate information, and in regard to the presence of any other forms of fructification no information of any kind. In the present paper I hope to be able to show that in both species a development, of what must apparently be regarded as spermogonia forms the starting-point of each annual cycle of development, and that in *Ravenelia sessilis* no less than five different forms of reproductive bodies are present,—spermatia, two forms of uredospores, and two forms of teleutospores.

Both species are extremely common in the neighbourhood of Calcutta, *R. sessilis* occurring on a large number of trees of *Albizzia Lebbek*, and *R. stictica* appearing everywhere on the leaves of *Pongamia glabra*.

Taking *R. sessilis* first and the period of complete defoliation of the host, which occurs in the beginning of the hot weather—as a rule during March—as a starting-point, we find the following series of phenomena manifesting itself. For the brief period during which leaves are entirely absent the only sites on the trees of *Albizzia Lebbek* on which any traces of the parasite are to be found are the dried-up and ripening pods, many of which remain adherent for a considerable period after the new crop of leaves has fully expanded. On these the remains of large teleutosporic clusters, and sometimes of mixed ones containing

both releutospores and uredospores are to be met with in varying numbers. The number of spores which are present in them is, however, small. On the dried dead pinnules beneath the trees, however, great numbers of teleutospores are to be found, together with smaller numbers of uredospores. When the new crop of leaves first makes its appearance and until the latter have fully expanded and the flowers have unfolded in numbers, there is no indication of the presence of the parasite. This state of things is, however, of very brief duration, and the leaves speedily become spotted with patches of spermogonia (Fig. 13, Plate I). Taking the phenomena presented by a tree in the year 1887 as an example, the following was the sequence of events: On March the 11th the tree was quite bare of leaves. On the 28th it was in full new leaf and just coming into flower, but showed no traces of a new crop of *Ravenelia sessilis*. On April the 3rd the new leaves were universally spotted, due to the presence of groups of spermogonia, and on the 17th the latter were beginning to dry up and blacken, while pustules of uredospores were appearing here and there at the edges of the groups.

The spermogonia make their appearance as small groups of minute, dull brown, circular, elevated points on somewhat discoloured and yellowish areas of the surfaces of the pinnules (Fig. 13, Plate I). The discoloration, as a rule, penetrates right through the thickness of the pinnules, and spermogonia appear at corresponding points on both upper and under surfaces. The size of the patches and the number of spermogonia which they contain vary considerably in individual instances. In groups of average size the spermogonial area has a diameter of about 0·5 mm., and is surrounded by a discoloured zone about 0·25 mm. in breadth. The number of spermogonia in a group varies from 1 or 2 up to 30, but, as a rule, 10 or 12 are present. In most cases they are arranged around a central point in circular fashion, several concentric circles being present in the larger groups, but in some instances the arrangement is irregular, or even almost linear. The number of groups on individual pinnules varies very considerably. In one case as many as 20 were present on the upper and 18 on the lower surface. Vertical sections through the pinnules show that the spermogonial areas of the laminæ are much thinner than surrounding ones, the patches being sunk below the level of the rest of the surface both on the upper and under sides of the leaves.

These thin spermogonial areas are throughout so densely occupied by fungal hyphæ that the normal structure of the pinnule is quite obscured. They stain very deeply with Spiller's purple. In the deeper, central portions of the lamina the hyphæ are arranged horizontally, so as to form a dense stratified mass. At the level of the epidermis the fungal tissue becomes more or less vertical, and masses of cells pass outwards between and around the compressed epidermal cells at particular points corresponding to the bases of the spermogonia. At the level of the outer surface of the epidermis dense masses

of erect twigs are given off, which gradually elevate the cuticle as they elongate. The peripheral twigs of each mass elongate much more than the central ones, and, growing up convergently around them, gradually form a definite bounding layer, which, as time goes on, gradually acquires a brown colour (Fig. 12, Plate I). The hyphæ of the included, cushion-like mass are slenderer than the peripheral ones. They do not elongate so much, but terminate in pointed extremities, which play the part of sterigmata and bear minute oval spermatia (Figs. 10, 11. Plate I). As maturation advances a definite opening arises at the apex of each spermogonium to permit of the discharge of the spermatia. Individual spermogonia vary greatly in size. Three which were situated side by side in one patch gave the following measurements, the first set of figures referring to diameter and the second to height—

1. = ·0759 × ·0198 mm.
2. = ·1320 × ·0330 „
3. = ·0660 × ·0297 „

They are always very broad in proportion to their height. The spermatia measure about $3{\cdot}25 \times 2{\cdot}75\mu$. After the spermogonia have matured they dry up and remain as minute black points in the centre of the patches of uredosporic pustules, which are next developed. Before they begin to dry up they usually present a peculiar glistening appearance, apparently connected with exudation of fluid from their orifices.

With the drying up of the spermogonia pustules of large uredospores begin to appear in the peripheral zones of the patches. They differ both in external appearance and in minute structure from the spermogonia. Instead of appearing as minute circular bodies opening at the apex by a definite orifice, they are of elongate form and open by mere irregular rupture. The latter phenomenon is due to the fact that they have not anything corresponding to the special bounding stratum of the spermogonia, and that the opening of the pustules is effected by mere rupture of the cuticle of the leaf, which has been gradually elevated by, and distended over, the growing mass of fungal tissue represented by the spores and the bed from which they arise.

The uredosporic pustules gradually increase in number, and form one or two rings around the central spermogonial areas (Fig. 14*a*, Plate I). Individual pustules sometimes become confluent, but the tendency to such an occurrence is never so great here as in the case of teleutosporic ones. There is no diffusion of infection over the general surface of this crop of leaves, each uredosporic group of pustules is situated immediately around a previously developed spermongonial one, and the number of groups of uredosporic pustules on any pinnule is hence determined by that of spermogonial ones. The deeper tissues of the leaf beneath the uredosporic areas are not nearly so much invaded by mycelium as they are in the central spermogonial areas. In fact, as is the case with the corresponding areas in the succeeding crop of leaves, it is often very difficult to demonstrate the presence of mycelial elements in them at

all. Around the surface of the epidermal cells, and between them and the cuticle, every uredosporic pustule shows a dense cushion, or disc, of small-celled fungal tissue, from which the stalks of the uredospores arise. The bed from which the uredospores arise is therefore in this species quite superficial, and, due to this, the stalks often remain adherent to the spores when detached by friction subsequent to the rupture of the cuticular covering of the pustules. An account of the structural details of the spores will be found in connection with the data regarding the subsequent crop of them.

No further development or diffusion of the parasite occurs in the leaves which are primarily affected, and at the end of the hot season it is represented simply by a set of groups of dried-up spermogonia, surrounded by varying numbers of concentrically arranged uredosporic pustules. With the onset of the rains a fall of leaf, almost, if not quite, as complete as that in the beginning of the hot weather, sets in, and a new crop of much larger leaves makes its appearance. This soon shows abundant evidence of the presence of the parasite, not, however, as in the case of the previous crop, by a preliminary eruption of spermogonia. These are entirely absent and groups of uredosporic pustules are developed directly (Fig. 14*b*, Plate I). These, both in their arrangement and in individual characters, differ somewhat from their predecessors. When they first appear they do not show any definite tendency to assume a concentric arrangement (Fig. 14*b*, Plate I), and to the end they never have it so strongly marked as those of the previous crop (Fig. 14*a*, Plate I). The groups are, moreover, naturally distinguished by the absence of the central groups of black points representing dried-up spermogonia. The individual pustules of this crop are considerably larger and more prominent than those of the preceding one; they are generally more or less circular in place of elongated, and they have a rich brown in place of a pale umber tint. They occur on both surfaces of the pinnules, but more abundantly on the lower ones. This is illustrated by the following figures. The pustules present on seven pinnules of a leaf were counted and found to number 126, but of this total only 37 were situated on the upper and 89 on the lower surfaces of the pinnules. They make their appearance at first in small numbers and quite isolatedly, but, as time goes on, the numbers increase and the pustules form distinct patches.

The mycelium corresponding with the pustules presents the same general features as that of the pustules of the previous crop, being relatively inconspicuous in the central strata of the leaf and forming dense sub-cuticular sporogenous beds. The size of the spores, when dry, appears to be the same for both crops, being about ·031 × ·015 mm., but, when wet, is possibly a little less in the spores of the second crop, in so far as length is concerned, probably due to slight differences in the nature of the epispore, which is somewhat thinner and less deeply coloured in them than in the spores of the previous crop. The stem is about ·005 mm. in thickness and of various lengths, up at any rate to ·06

mm. In form the spores are obovate. They are of a rich, golden-brown colour over the greater part of the surface, and deep brown over the apex, due to the increased thickness of the epispore here (Fig. 9, Plate I). Over the upper half of the spore the epispore is thickly tuberculate, and below this scattered tubercules are also present. About half-way up a series of circular pores form a band around the body of the spores (Fig. 8*a*, Plate I). Each spore contains a large, pale nucleus, surrounded by a mass of cytoplasm, which, in the case of the first crop, is often more or less coarse and lumpy in texture, but in that of the second one is finely granular.

When an infected leaf fades, the tissue in, and immediately around, the pustular patches retains its brilliant green colour long after the rest of the surface has become bright yellow, and the same phenomenon holds good in regard to teleutosporic patches. This indicates the fact, which is borne out by microscopic examination of sections, that there is no injury to the vitality of the tissue-elements of the host within the specially infected areas. On the contrary, there is very manifest evidence of protoplasmic hypertrophy, especially in the epidermal cells, and the parasitic and host elements appear to hold a relation to one another very similar to that which the fungal and algal elements hold in many lichen thalli. Within the green pustular areas the chlorophyll corpuscles are plump and well preserved, and there is manifest excess of colourless cytoplasm specially in the epidermal cells. In the surrounding yellow areas the cytoplasm generally is very scanty, and the chlorophyll corpuscles are represented by mere shrunken, yellowish granules. It might at first sight appear that the detachment and destruction of cuticular tissue connected with the maturation of the pustules must necessarily produce an injurious effect on the subjacent tissues of the host. This is not the case, however, due, no doubt, to the fact that the exposed surfaces are everywhere covered by a dense continuous stratum of small-celled fungal tissue, from which the spores originate and which replaces the cuticle in protective function. As will be shown in the case of *R. stictica*, where such a protective surface layer of fungal tissue is absent, desiccation and death of the host tissues within the pustular areas does eventually take place.

For some time only pustules of the character described above, containing large uredospores, present themselves, and then others of mixed character begin to appear. In the first form of mixed pustules which appear the pustules contain, in addition to the large uredospores, varying numbers of much smaller ones (Fig. 16, Plate I). In some cases these are present in enormous numbers, but they never occur alone, being always associated with either uredospores or teleutospores, or with both. They are of narrow, oval form, measure about $8 \times 2\ \mu$, and are almost colourless, having at utmost a pale, bluish green tinge (Fig. 8 *b*, Plate I). They contain a finely granular protoplasm and a distinct, pale nucleus, which stains deeply with Spiller's purple. They are connected by relatively long

pedicles with the same bed of small-celled fungal tissue from which the large uredospores and teleutospores which they accompany arise. In order to distinguish them from the large uredospores they may be described conveniently as microspores.

It is not for some time later, and only towards the close of the rains, that teleutospores begin to appear. They appear first in the outer pustules of the groups on the upper surfaces of the pinnules which have gradually replaced the original isolated pustules. On the under-surfaces of the pinnules the number of pustules remains throughout limited, and they do not here form concentric groups, but either remain solitary or form small irregular patches. This is probably due to the uredospores falling away from the pinnules, and not remaining in the neighbourhood of the parent pustules to the same extent which they do on the upper surfaces, and, correlated with it, we find a much smaller development of teleutospores taking place. At first teleutospores only appear in small numbers in pustules otherwise occupied by uredospores, but a rapid increase takes place in their numbers in subsequently developed pustules, and within a short time pure teleutosporic pustules, and pustules containing teleutospores and microspores only, make their appearance. Still later, very few uredosporic or microsporic pustules are developed on the upper surfaces of the pinnules, while pure teleutosporic ones go on appearing in constantly increasing numbers up to the time when the leaves fade and fall. Uredosporic pustules continue, however, to form the characteristic feature on the lower surfaces throughout.

During the earlier portion of the period in which teleutospores are formed no less than six kinds of pustules are present in large numbers. These are :—

1. Pustules containing uredospores.
2. „ „ „ and microspores.
3. „ „ teleutospores.
4. „ „ „ and uredospores.
5. „ „ „ „ microspores.
6. „ „ „ „ and uredospores.

When occurring in mixed pustules teleutospores appear, on the whole, to be more frequently associated with microspores than with uredospores.

As in the case of the uredosporic fructification the deep-seated mycelium of the teleutosporic one is usually very difficult to demonstrate satisfactorily in the pinnules. Where the pods are affected, however, excellent specimens can often be obtained, showing jointed mycelial filaments forcing their way between the cells of the host and sending short-rounded haustoria into their cavities (Fig. 15, Plate I). A dense mass of small-celled fungal tissue is developed subcuticularly at the site corresponding to each pustule, and at intervals this is continued downwards between the cells of the epidermis to join the filamentous

mycelium below (Fig. 2, Plate II). This cushion of small-celled tissue becomes clothed by a stratum of short, erect, often somewhat clavate cells (Fig. 17, Plate I). These ultimately give rise to the spores, but the development does not take place simultaneously over the whole sporogenic layer. On the contrary, successive crops of spores are developed until the layer is gradually exhausted. At any point where spore formation is about to occur two or three adjacent cells elongate, become manifestly clavate, and ultimately adhere to one another at their free extremities. The latter next become shut off by transverse partitions, and are further sub-divided into two or more portions by vertical ones. The entire body at this stage consists of several separate stems, and a common broad head, composed of a number of closely adherent cells, varying in number from four to nine, or even twelve.

Each of these cells next gives origin to two or three terminal cells, which also adhere to one another by their lateral surfaces, and the head thus becomes more or less hemispherical. The distal cells of the head now grow much more rapidly than the basal ones, assuming an obconical form and spreading out so that the entire mass becomes flatter than at first (Fig. 18, Plate I). It now consists of a concavo-convex stratum of large distal cells, borne on a somewhat convex mass of basal cells. Whilst these changes are taking place in the head, the cells of the stalk elongate, and, as this elongation appears to be in great part due to the upward pressure of neighbouring, younger spore-elements on the head, they become greatly attenuated in doing so. The outer surfaces of the external stratum of basal cells of the head have gradually, as the latter expands, come to be directed downwards, and they each now begin to develope a protrusion towards their upper, or, as it now is, outer extremity (Fig. 4, Plate II). A series of short sacs thus makes its appearance all round the head on its under-surface, and, as these gradually elongate, the so-called cysts are formed. The upper, convex surface of the head now becomes gradually invested by a thick continuous stratum of cuticle of a deep brown colour (Fig. 3, Plate II). It is not tuberculate, but simply irregularly thickened over the greater part of the surface, but a single row of true short tubercles is situated along a line corresponding with the outer margin of the spore, where the outer and inferior surfaces of the external row of cells meet (Figs. 5, 6, Plate I). Even in very old spores there is not the slightest tendency to separation of the constituent cells, the continuous thick cuticular epispore binding them all together, so as to resist pressure and friction very strongly, so that when rupture does occur it merely takes the form of irregular fissuring. In this they differ from the spores of *R. aculeifera*, as described by Cooke[1], and from those of a yet undescribed species occurring on *Phyllanthus emblica*, of which I published figures in 1871[2]. As maturation

[1] "The Genus Ravenelia," by M. C. Cooke, M A.—Journal of the Royal Microscopical Society. Vol. III, 1880, p. 384.

[2] Appendix B, Annual Report of the Sanitary Commissioner with the Government of India for the year 1870. Calcutta, 1871.

advances the stem-cells and the basal cells dry and shrivel up, save the cystic protrusions of the latter, and ultimately the head is detached from the stem, usually due to the excessive upward pressure of younger spores. The stem cells are now once more set free from one another and remain as a series of long hair-like processes on the surface of the pustule (Figs. 17, 18, Plate I). The length of these hairs is very considerable, in some cases being as much as ·066 mm., and renders the specific name of the species very inappropriate. The epidermal cells of the host beneath the pustules are specially rich in protoplasmic content, and are also of somewhat smaller size than the corresponding cells elsewhere, either due to pressure or to processes of proliferation similar to those often occurring in the case of the algal elements of a lichen. The subepidermal cells beneath the pustular areas are also characterised by excess of protoplasmic content, although not to such a high degree as the epidermal ones.

The mature detached teleutospores, when first separated from their stalks, consist of flattened concavo-convex, more or less circular masses of cells. The convex surface is covered by a thick, deep brown epispore, and surrounded by a row of short tubercles (Figs. 5, 6, Plate I). The under-surface presents a marginal, elevated, somewhat convex rim, corresponding with the true outer, but now under, surfaces of the marginal row of spore-cells. Within this is situated the row of cystic protrusions of the outer portions of the peripheral basal cells, which in the normal fresh spores pass more or less vertically downwards as a fringe of short ,colourless, highly refractive blind tubes, but which in dried specimens very frequently become flattened out, so as to form a horizontal frill around the spore (Figs. 1, 2, 3, Plate I). The central deeply-concave area of the under-surface corresponds with the shrivelled central basal cells and central portions of the peripheral ones (Fig. 7, Plate I).

The cystic protrusions of the peripheral basal cells do not persist beyond a certain period in the mature spores. Their function is apparently to facilitate the adhesion of the spores to surfaces with which they may come in contact, and with the rest of the basal cells they soon disintegrate and disappear. The spores, therefore, at different periods present very different appearances. Newly matured ones show the cysts very clearly, while in many old ones not a trace of them remains. Intermediate forms occur, showing the cysts in various stages of disintegration. The cysts at an early stage are, as previously mentioned, strongly refractive and appear plump and full. Subsequently, they become much dilated, and at the same time their contents shrivel up into threads and their walls become very thin, save along their lateral surfaces. Ultimately the thin portions of the walls disappear, and the thicker ones persist as a series of fine filaments fringing the spores and looking not unlike fine mycelial threads. Other thread-like elements also are present in some cases, due apparently to persistent remnants of the cell contents remaining after the solution of the walls. When the cysts and basal cells have disappeared, the spores, when viewed from

beneath show a deep central depression corresponding with the bases of the spore-cells (Fig. 6, Plate I). Immediately around this an imperfect cuticular ridge is present, indicating the site where the outer surfaces of the peripheral basal cells were attached to the outer edges of the bases of the corresponding spore-cells (Fig. 5, Plate I). External to this is the zone corresponding with the extero-inferior surfaces of the peripheral spore-cells, surrounded externally by the line of tubercles separating these surfaces from the true apical areas of the same cells.

The size of individual spores varies very greatly, due to the varying number of stems and basal cells from which they originate. Those of average size and regular form measure about ·085 mm. in diameter and about ·023 in height, but in some cases they may attain a diameter of ·1 mm. and a height of ·04 mm. Others again are very small. The size is determined by the number of spore-cells present, the latter varying very little in magnitude, as a rule. The central cells at their upper extremities present hexagonal, pentagonal, and rarely quadrangular faces. The marginal ones are pentagonal or quadrangular, and are somewhat larger, or at all events broader, than the central ones, giving diameters of about 19·8μ × 16·5μ to the surface of the thick epispore, which is here about 3μ in thickness, while the central cells are about 16·5 × 14·8μ. Some spores contain as few as seven cells, and others as many as thirty-seven or more. Each mature cell contains a finely granular protoplasm, including a clear nuclear area and in many cases one or more oil globules.

All attempts at artificial cultivation of teleutospores have as yet failed to give any results. The uredospores, on the other hand, germinate freely in infusion of the leaves of the host. Several attempts at artificial infection by means of teleutospores have been made, but only in one case was there any apparent result. This, however, may have been due to the fact that the seedling plants which were supplied for experiment as specimens of *Albizzia Lebbek* eventually turned out not to belong to that species, but to *Albizzia procera*. In the case in which a result appeared the pinnules of a healthy seedling were smeared with water containing an abundance of teleutospores, and shortly afterwards showed two patches of spermogonia, agreeing precisely in character with those normally developed on *Albizzia Lebbek*.

Late in the season, and shortly before the fall of the leaves, a second and very distinct form of teleutospore often occurs in the persistent uredosporic pustules on the under-surfaces of the pinnules (Fig. 1, Plate II). They arise by simple stems about ·059 mm. in length from the same bed of small-celled tissue as the surrounding uredospores of the pustule. The spore is rounded and apparently composed of four cells, surrounded by a common thick epispore, of dark sepia colour, and beset with a number of peculiar olive-green processes, about 10μ. in length, arising by dilated bases and terminating above in stellate expansions (Fig. 4, Plate I). The body of the spore without the processe

measures about 33×30μ. Such spores never occur independently. They are always associated with normal uredospores, and as they appear to rise from the same sporogenic tissue as the latter do, they must provisionally be regarded as a second form of teleutospore of *R. sessilis*.

The series of events occurring in the annual cycle of development of *R. stictica* is essentially parallel to that present in the case of *R. sessilis*, but differs in regard to certain details, which are partly intrinsic and partly dependent on differences in the characters of the host plant. As in the case of the other species the cycle may be regarded as commencing with the appearance of the new crop of leaves on the host subsequent to the complete defoliation which takes place at the beginning of the hot weather. The leaves which fall at this time are universally coated beneath by a dirty brownish stratum, consisting of innumerable teleutospores and a sprinkling of uredospores. The trees are bare of leaves for a few days only, and then become covered by a crop of brilliantly green new ones, and these very soon show signs of infection in the appearance of minute, dull ochreous spots, consisting of groups of spermogonia (Fig. 8, Plate II). On the upper surfaces of the leaves the spots are slightly elevated, and on the under ones correspondingly depressed. Each patch, as in the case of the corresponding fructification in *R. sessilis*, passes right through the leaf, the tissues of the host being interwoven with a dense web of mycelial filaments connected on either side with cushions of sub-cuticular, small-celled elements. The groups vary in size greatly, but large ones may measure as much as ·625 mm. in diameter. When the spermogonia first appear they show as minute hard whitish prominences on a surface of apparently unaltered host tissue. Afterwards they gradually become brownish peripherally, with a central ochre-coloured area, where they ultimately open. Finally, pale yellowish masses of spermatia escape from the orifices, apparently involved in a mucoid basis (Fig. 10, Plate II). By this time the spermogonial areas of the lamina have become somewhat deeper green than the rest of the surface, and are somewhat elevated or depressed according to the side of the leaf on which they are situated. A narrow yellowish peripheral zone is sometimes recognisable around the patches. Still later, and when uredosporic pustules are beginning to break out peripherally, the spermogonial areas become brownish. The spermogonia on the upper surfaces of the leaves are much more conspicuous than those on the lower ones, due to the relatively small development of uredosporic pustules which takes place around them.

The deeper tissues of the laminæ within the spermogonial areas are everywhere interwoven by delicate, septate mycelial filaments with intracellular haustoria, which at the level of the epidermis become continuous with masses of cells, passing upwards around the cells of the host and uniting to form dense cushions of small-celled tissue constituting the bases of the spermogonia (Figs. 9, 10, Plate II). From these, dense masses of erect, more or less convergent filaments arise,

forcing the cuticle in front of them as they elongate. The peripheral ones are considerably longer than the central ones and ultimately acquire a brownish colour. They converge around the central ones and form a definite bounding stratum. Oval spermatia are developed from the tips of the shorter central filaments and accumulate in masses until, on the rupture of the cuticle and the separation of the apices of the filaments of the bounding stratum, they are enabled to escape in masses upon the surfaces of the leaves (Fig. 10, Plate II).

The spermogonia are somewhat smaller than those of *R. sessilis*, having an average diameter of ·0766 and a height of ·0255 mm. The spermatia, on the other hand, are larger than in the other species, giving diameters of $3 \times 6\mu$.

Another feature distinguishing the spermogonial fructification of *R. stictica* from that of *R. sessilis* lies in the nature of the bases of the spermogonia. The spermogonial beds of small-celled tissue are not so sharply defined and limited to a sub-cuticular site as those of *R. sessilis* are, as the amount of fungal tissue around the epidermal cells is so great, in many cases, as to displace and conceal the latter very considerably. In some cases the displaced cells appear to contain complex haustoria, and in others, especially in the areas intervening between the bases of neighbouring spermogonia, they seem to be aborted, discoloured, and shrunken. In many places the epidermal tissue appears to be entirely replaced by the masses of small-celled fungal tissue, which thus come to rest directly on the pallisade cells when related to the upper surface of the lamina. When this is the case the pallisade cells are often greatly malformed, being enlarged, rounded, and seemingly occupied by haustoria.

The uredosporic fructification begins to make its appearance very shortly after the spermogonial one. The patches are at first small and distinctly annular, consisting of elongated, often confluent pustules, surrounding the spermogonial areas (Fig. 11, Plate II). They afterwards come to vary greatly in size and may become irregular in outline. The definite annular arrangement is soon obscured as the masses of spores fall off and accumulate within and around the spermogonial areas. The size attained by some patches is very considerable. One which was measured gave diameters of $3{\cdot}5 \times 2{\cdot}25$ mm. The pustules are developed much less conspicuously around the spermogonial areas of the upper surfaces of the leaves than around those of the lower ones. In many cases, indeed, they are entirely absent, and when present they never form large groups, but merely form a single, often imperfect ring. The number of uredosporic patches varies with that of the spermogonial ones, but may be very considerable, fifty or sixty being frequently present on the under-surface of a single leaf. Up to a certain time the spermogonio-uredal areas appear as conspicuous green patches when the leaf on which they are situated fades prematurely. When this does not occur, however, they ultimately dry up and become brown and brittle, readily breaking up and leaving no traces behind, save perforations in the lamina.

Before passing on to details regarding the mycelium and spores of this

fructification, it is necessary to describe certain of the structural features of the host tissues. The leaves of *Pongamia glabra* are characterised by the presence of a double row of pallisade-cells beneath the superior epidermis. Beneath the deep row of pallisade-cells is a stratum characterised by the presence of large, irregularly rounded cells, between which and the inferior epidermis one or two rows of delicate elongated cells, with their long axis vertical to the epidermis, and leaving large intercellular spaces, build up a loose tissue.

Dense masses of delicate jointed mycelial filaments are visible at the level of the deeper pallisade-cells and of the stratum of large rounded cells immediately beneath them, and these, in the case of the majority of pustules—of all those appearing on the inferior surfaces of the leaves—are continuous with solid masses of small-celled tissue which surround and displace the deeper portions of the subepidermal tissue to form the sporogenic bases of the pustules. The stalks of the uredospores thus originate relatively far from the surface and pass up between the superficial portions of the subepidermal tissue, while the spores themselves are developed beneath the epidermis, which they gradually elevate, and ultimately rupture, as they increase in size and number. Due to the deep origin of the stalks the spores might readily be supposed to be sessile, as, when detached naturally or by means of friction, they, of course, very seldom carry any portion of their stalks along with them, and it is only in sections in which they remain in their normal position that their true character becomes visible. Where uredosporic pustules appear on the upper sufaces of the leaves the spores are developed subepidermally also, but the sporagenic beds of small-celled tissue do not lie so deeply as in the previous case, being apparently developed between the under surface of the epidermis and the outer extremities of the pallisade-cells.

The young uredospores are oval, but, as they mature, they become broader, and many of them are ultimately almost spherical (Fig. 7, Plate II). Two of average size were measured while dry, and gave diameters of ·0195 × ·0195 and ·021 × ·0195 mm. A slight increase in size usually occurs when they are immersed in water. They are yellowish brown, and the epispore is everywhere thickly tuberculate, save at one large rounded area, apparently corresponding with the site of insertion of the stalk. Just as in the case of *R. sessilis* there is no evidence of general diffusion of infection over the surfaces of the leaves at this period, the groups of pustules being limited to the immediate neighbourhood of the spermogonial areas which they surround, and towards the close of the hot weather the fructification is almost, if not entirely over, the sites of previous spermogonio-uredal patches being merely indicated by brown desiccated spots, or by perforations in the laminæ resulting from their disintegration.

Only a very limited fall of old leaves and evolution of new ones accompanies the onset of the rains in *Pongamia glabra*, and for some time the parasite makes no sign of its presence. Generally diffused infection of the deeper tissues must,

however, be taking place, for, after the lapse of a few weeks, the under-surfaces of the leaves, both old and new, become gradually coated with a thin, brownish stratum due to universally distributed eruption of minute pustules containing uredospores and teleutospores. There is in this crop not a trace of spermogonia. Sections of the laminæ show them to be everywhere penetrated by mycelial filaments. At points corresponding with the bases of the pustules, such filaments are present in great abundance and become continuous with masses of cells which, as in the case of those of the uredosporic fructification of the previous crop, are situated in the intercellular spaces between the deeper portions of the subepidermal cells of the host (Fig. 12, Plate II). From these masses of cells teleutospores in large numbers, and a certain number of uredospores, arise. The pustules are always of very small size, only yielding a small number of spores each, but, as they are everywhere present, the quantities of spores, and specially of teleutospores which they furnish, are enormous. The uredospores resemble those of the previous crop, and, like them, have long stalks, which pass up between the cells of the subepidermal tissue and give origin to the spores beneath the epidermis, which is first elevated and stretched, and finally ruptured, as they increase in size. The development of the teleutospores is essentially similar to that of those of *R. sessilis*. As in that species, each spore is the product of several distinct stems which unite terminally and develop a mass of basal cells from which the true spore-cells are produced (Fig. 13, Plate II). The number of basal cells and spore-cells always, however, remains comparatively limited, and the basal cells do not give off any special cystic protrusions. Perfectly fresh mature spores are, however, not devoid of cystic appendages, the entire bodies of the basal cells becoming dilated and taking the place of the special cysts. Each detached spore at this time is thus seated on a very delicate cushion of very tenuous cysts, which in some cases carries a short portion of the stalk attached to it inferiorly. The cysts are very perishable, and they never show the brilliant refraction of the fresh cysts in *R. sessilis*. As in the case of the cysts of that species fine mycelioid radiant filaments, due to persisting portions of the cell-walls, often form fringes around the base of the spores after the cysts themselves have disappeared.

The teleutospores vary very greatly in size, due to the very different number of constituent cells which is present in different instances. In some spores only five cells are present, while others may contain as many as nineteen or twenty. The spores never attain magnitudes equal to those of large teleutospores of *R. sessilis*. On an average they have a diameter of about ·0626 mm. and a height of ·0198 mm. They are of a deep golden brown colour. The epispore over the upper-surface is thickly tuberculate (Fig. 5, Plate II), and the marginal cells are also provided with a single row of large spines, situated, like the row of tubercles around the teleutospores of *R. sessilis*, along the line where their inferior and outer surfaces meet. The size of the individual spore cells varies

with their position as in *R. sessilis*, the marginal ones, as a rule, being larger than the central ones. The central ones superiorly have pentagonal or quadrangular, rarely hexagonal faces, and give diameters of about $18{\cdot}1 \times 16{\cdot}5\mu$. The marginal ones are quadrangular or pentagonal, and are about $19{\cdot}8\mu$ in transverse diameter and from their outer to their inner margins. After the basal cells have entirely disappeared the external spore-cells still show the site of attachment of their outer edges by means of a line of projecting cuticular ridges (Fig. 6, Plate II). External to this line the epispore is smooth up to the line of spines separating the inferior and extero-superior surfaces of the spore, and beyond this it becomes tuberculate. Mature spores, after the disintegration of the cystic basal cells, have a concavo-convex form, the convexity being relatively somewhat greater than in the case of the spores of *R. sessilis*, due to the smaller number of cells present (Fig. 5 *b*, Plate II). There is no tendency to separation of the cells even in very old spores, either naturally or as the result of pressure or friction. Only a limited number of spores is developed in each pustule due to the small extension of the sporogenic issue in any case, and, as they are developed successively, as in the case of those of *R. sessilis*, it is rare to find any pustule with more than one mature spore connected with it. Due to the deepsite of the sporogenic tissue and the inclusion of the stalks of the spores within the host-tissues, the pustules never show hair-like processes, like those occurring on the superficially seated pustules of *R. sessilis*.

The principal points demonstrated by these observations are the following :— *1st*, that the cycle of development in Ravenelia is, save in the absence of an Æcidial fructification, of the type normal to the Uredineæ, beginning with an eruption of spermogonia and terminating with the development of teleutospores ; *2nd*, that the cysts are merely the altered basal cells from which the spore cells originate ; *3rd*, that the mycelioid filaments which have been described as connected with the spores in certain cases, for example in *R. macrocystis*, B. & Br.,[1] are merely persistent portions of cysts.

So long as the presence of the basal cells was not recognised, it was very difficult to account for the presence of the cysts, but when once it is, and when we know that the spore-cells are not directly attached to the summit of the stalk their origin becomes extremely simple. It is, however, not to be wondered at that the presence of the basal cells should have escaped the notice of observers whose material consisted solely of old dried specimens, as they are so delicate and fragile as either to disappear entirely in the course of drying, or, at utmost, to persist in very imperfect condition. Judging by the figures, however, an exception presents itself in the case of *R. glandulæformis*, B., for here the elongated cysts are depicted as intervening between the summit of the stalk and the mass of spore-cells.[2] The recognition of basal cells also enables

[1] Journal of the Linnean Society: Botany, Vol. XIV, p. 93.

[2] M. C. Cooke, M.A., *op. cit.*

us to understand how such great masses of cells as are present in such teleutospores as those of *R. sessilis*, come to be connected with such slender stalks, which remained an obscure point even when the complex nature of the stem was recognised, but the spore-cells were regarded as taking origin directly from it. Finally, it is clear that, in dealing with dried specimens of detached spores, the presence or absence of cysts or stalks cannot be regarded as of specific importance, seeing that in one and the same species cysts may be present at one time and absent at another, and that stems of very considerable length may be present without having any tendency to adhere permanently to the basal cells, more especially in cases such as that of *R. stictica*, in which they remain throughout buried among the tissues of the host.

D. D. CUNNINGHAM.

CALCUTTA;
23rd October, 1888.

PLATE II.—FIG. 4. Portion of a teleutosporic pustule of R. resiliis, showing development of teleutospores and cysts, × 370.

" 5. Teleutospores of R. stictica; *a*, upper surface; *b*, profile; *c*, sectional view, × 370.

" 6. Under surface of teleutospore of R. stictica after disappearance of basal cells, × 690.

" 7. Uredospores of R. stictica, × 690.

" 8. Spermogonial patch of R. stictica, × 77.

" 9. Vertical section through two spermogonia of R. stictica, showing the bed of small-celled tissue from which they arise, × 370.

" 10. Section of spermogonium of R. stictica, showing discharge of spermatia, × 690.

" 11. Uredosporic pustules of R. stictica surrounding old spermogonial patch, × 50.

" 12. Vertical section through a leaf of Pongamia glabra including a young teleutosporic pustule of R. stictica, showing deep site of sporogenic bed and of origin of stalks of teleutospores, × 370.

" 13. Young teleutospore of R. stictica, showing sporogenic bed, stem cells, basal cells, and spore cells, × 690.

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„ 5. Teleutospores of *R. stictica*; *a*, upper surface; *b*, profile; *c*, sectional view, × 370.

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„ 13. Young teleutospore of *R. stictica*, showing sporogenic bed, stem cells, basal cells, and spore cells, × 690.

Description of the Plates.

PLATE I.—FIG. 1. Fresh teleutospore of *R. ressiliis*, with the cysts still persistent and forming a fringe due to pressure, × 180.

" 2. Fresh teleutospore of *R. ressiliis* in half profile view, × 360.

" 3. Fresh teleutospore of *R. ressiliis* in profile view, × 360.

" 4. Second form of teleutospore of *R. ressiliis*, × 690.

" 5. Portion of the under surface of a teleutospore of *R. ressiliis*, showing marginal tubercles, and cuticular ridges indicating the line of attachment of the basal cells, × 690.

" 6. Teleutospore of *R. ressiliis* after disappearance of the basal cells viewed from below, × 690.

" 7. Diagram of structure of an attached teleutospore of *R. ressiliis*, showing stem cells, basal cells, and spore cells, and origin of cysts.

" 8. A uredospore and two microspores of *R. ressiliis* after treatment with Spiller's purple, × 690.

" 9. Uredospores of *R. ressiliis*; *a*, fresh and in air; *b*, after immersion in alcohol and water, × 900.

" 10. Portion of a spermogonium, showing part of the wall and stelgmata and spermatia projecting above it, × 690.

" 11. Vertical section through a spermogonium, × 370.

" 12. Section showing wall of spermogonium, × 370.

" 13. Patch of spermogonia of *R. ressiliis*, × 50.

" 14. Pinnules of *Albizzia Lebbek*; *a*, spring leaf, with spermogonia and uredal pustules; *b*, Rains' leaf, with uredal pustules. Natural size.

" 15. Mycelium of *R. ressiliis* in the tissue of a pod of *Albizzia Lebbek*, × 660.

" 16. Pustule containing uredospores and microspores, × 150.

" 17. Portion of a young teleutosporic pustule, showing origin of stems of spores, × 600.

" 18. Portion of a teleutosporic pustule, showing developing spore, sporogenic bed, and hairs formed of stalks of detached spores, × 660.

PLATE II.—FIG. 1. Vertical section through part of a pustule of *R. ressiliis*, showing uredospores and second form of teleutospores arising from the same sporogenic bed, × 370.

" 2. Portion of a section of a pod of *Albizzia Lebbek*, showing the sporogenic bed of a teleutosporic pustule of *R. ressiliis* detaching the cuticle from the epidermis, × 660.

" 3. A teleutospore of *R. ressiliis* still attached to the sporogenic bed, × 370

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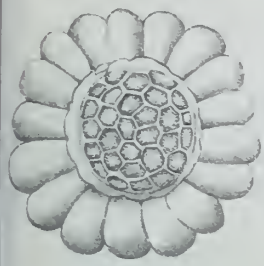


Fig. 1×180.

Fig. 2.×360.

Fig 3 ×360

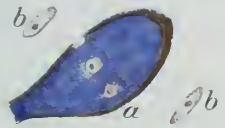
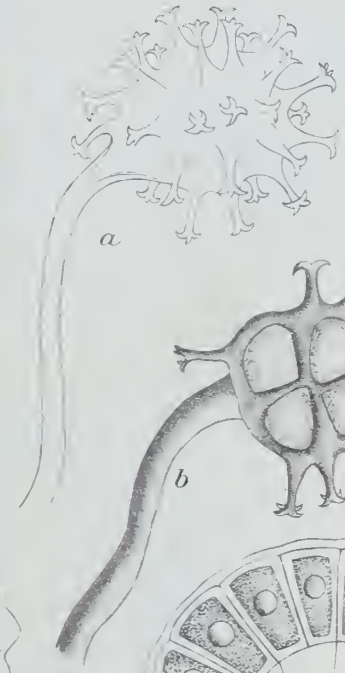


Fig. 8×690.



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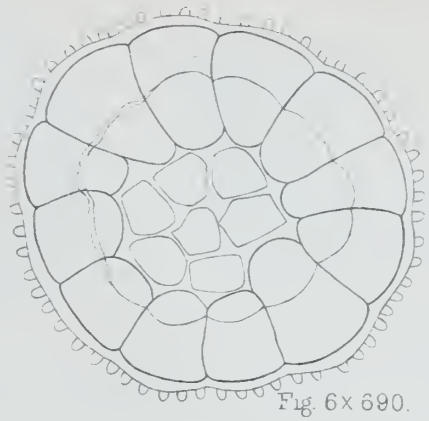


Fig. 6×690.

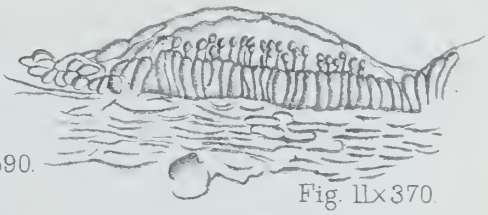


Fig. 11×370.

Fig. 12×370.

Fig. 9×900.

Fig. 5×690

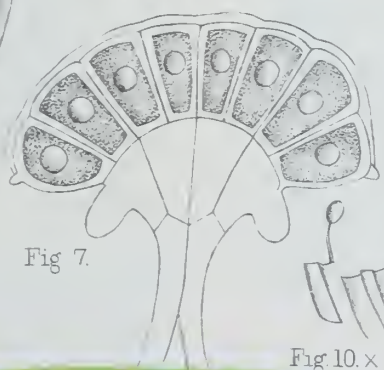


Fig 7.

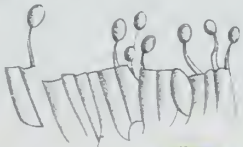


Fig. 10.×690.

Fig. 14.

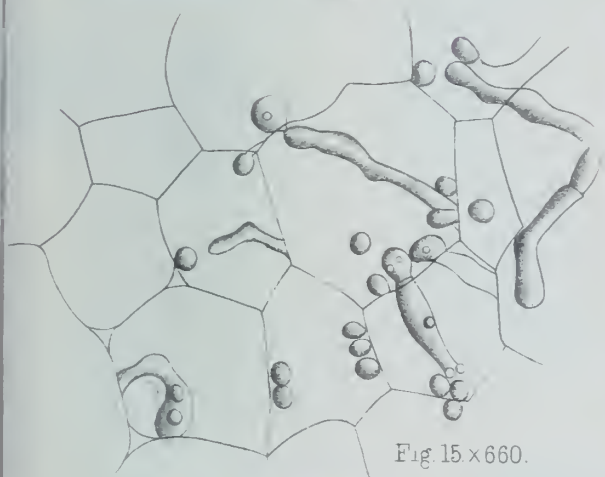


Fig. 15.×660.

Fig. 13.×50.

Fig. 16.×150.

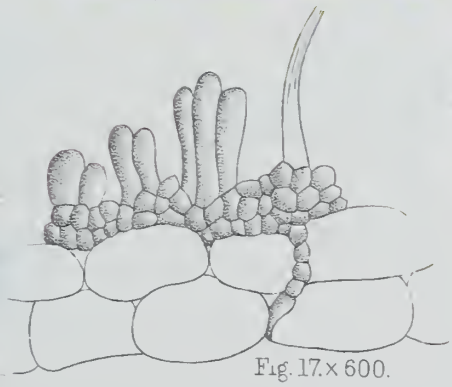


Fig. 17.×600.

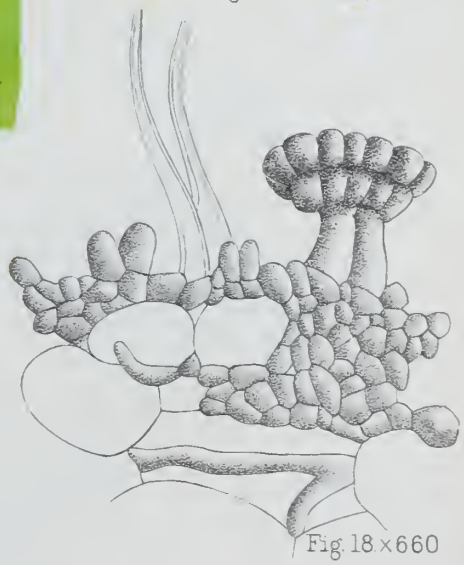


Fig. 18.×660

RAVENELIA SESSILIS.

Lithographed at the Survey of India Offices, Calcutta, July 1888.

Fig. 1.×370.

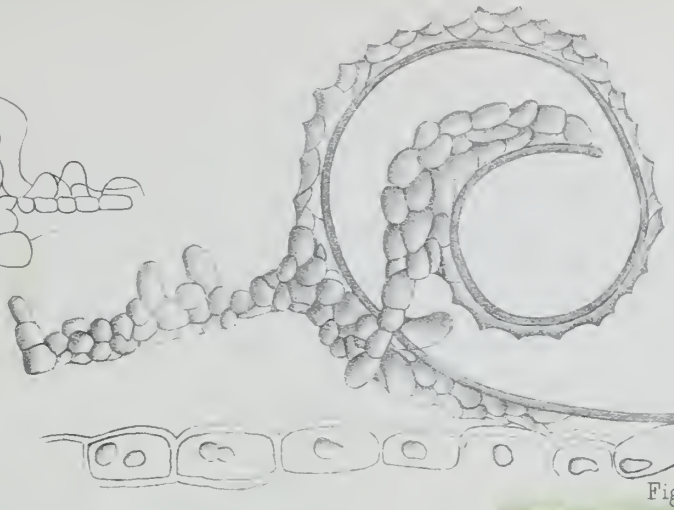


Fig 2.×660.

Fig 3.× 370.

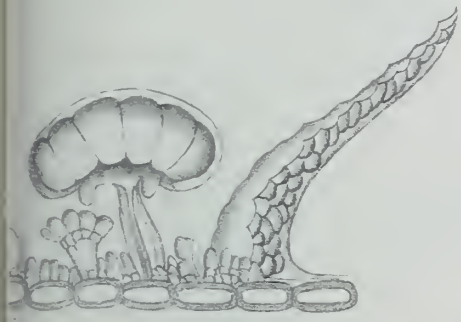
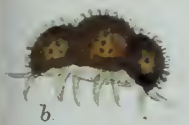
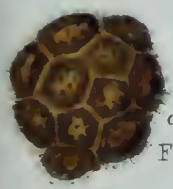


Fig. 4.×370.



b.

c.



a

Fig. 5.×370.

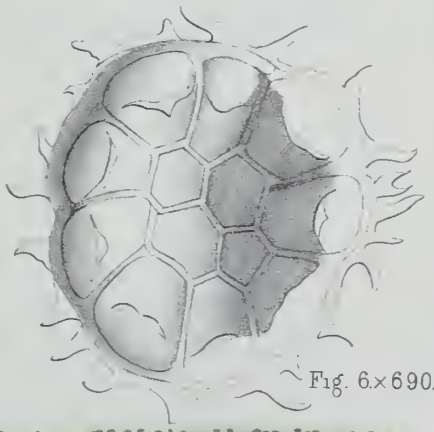


Fig. 6.×690.

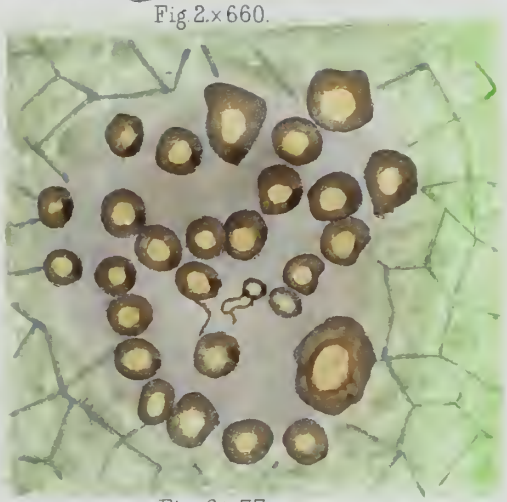


Fig. 8.×77.

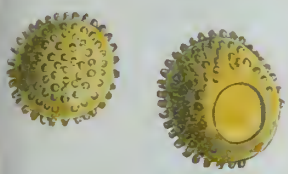


Fig. 7.× 690.

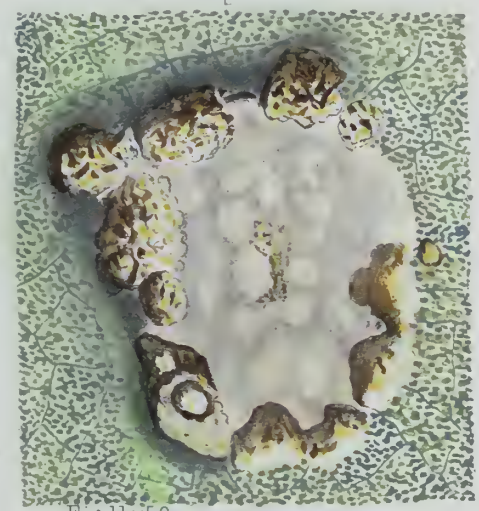


Fig. 11.×50.

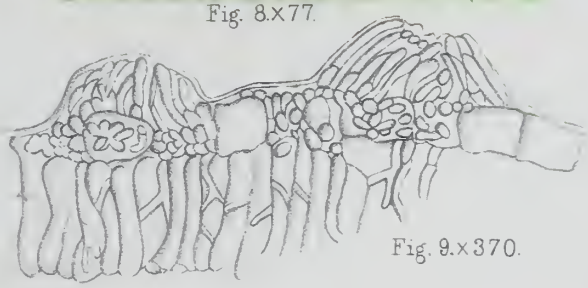


Fig. 9.×370.

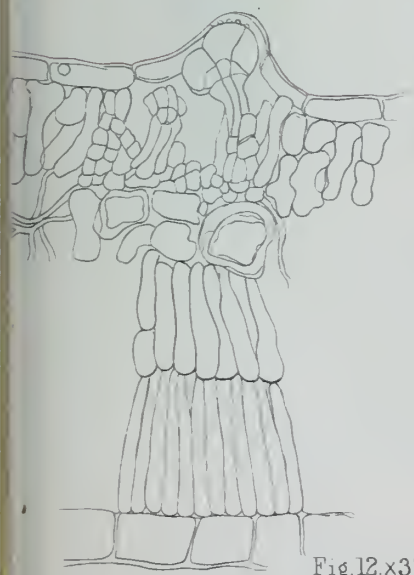


Fig. 12.×370.

Fig 13×690.

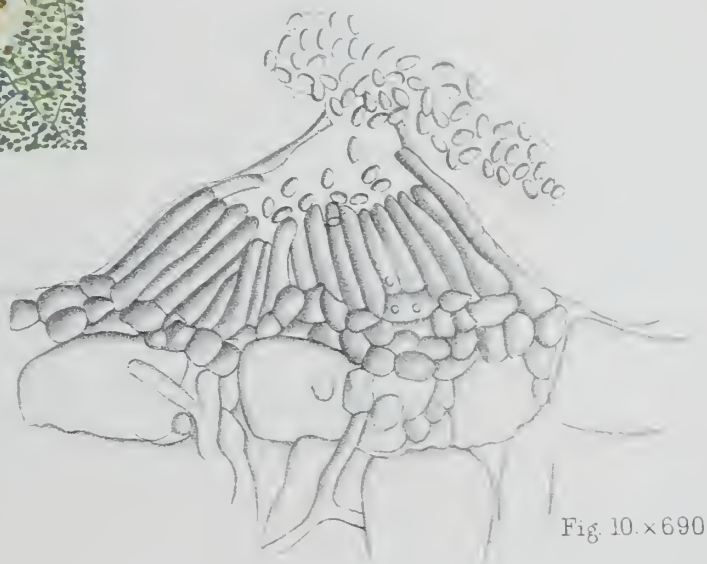


Fig. 10.×690.

FIGURES 1 - 4. RAVENELIA SESSILIS, B.

,, 5-13. RAVENELIA STICTICA, B. & Br.

Lithographed at the Survey of India Offices, Calcutta, October 1888.

On the Life-history of a new Cæoma on *Smilax aspera*, Linn.;

BY

A. BARCLAY, M.B.,

BENGAL MEDICAL SERVICE.

My attention has been directed for some time past to an interesting Uredine, which occurs on *Smilax aspera*, Linn., in Simla (North-West Himalayas), but I have hesitated thus long in publishing a description of it, because I hoped to have been able to complete some gaps in its life-history, and because, for reasons which will presently appear, I felt some uncertainty as to whether the aecidial form was really such, or only an abnormal uredo form. My doubt on this latter point has, however, been removed since I had the opportunity lately of consulting Professor R. Hartig of Munich upon it, and of showing him my preparations, and I take this opportunity of expressing my best thanks both to him and to his assistant Freiherr von Tubeuf, not only for the kind interest they took in this research, but also for the extreme kindness and courtesy with which they received me during my stay in Munich. Professor Hartig quite agrees with me in regarding this hitherto somewhat doubtful form as a true Aecidium, and it now only remains for me to explain why I do not still delay publishing an account of it, until I have been able to clear up some remaining doubtful points in the life-history of the parasite. I have determined to delay no longer, because I cannot now foresee when I may be able to take up the investigation again, and because the facts I have already obtained appear to me to be sufficiently interesting to warrant a publication of them as they are.

The features of special interest in this Uredine are, (*a*) that it is apparently the first complete autœcious Cæoma yet described; (*b*) the peculiar form of germination exhibited by the teleutospores; (*c*) the peculiar way in which the aecidiospores are abstricted and shed; and (*d*) the suggestive resemblance exhibited by the teleutospores to the genus Gymnosporangium. But, before entering into these points, it is necessary first to describe the fungus generally—

General characters and natural history of the Fungus.—It will be most

convenient to begin with the aecidial stage found towards the end of June, or in July, when the host, after the setting-in of the rains, throws out its new shoots and leaves. At this time, in certain definite localities only, although the plant is widely distributed all over the region, these newly-developed leaves, which are readily distinguishable from the older leaves of the previous year's growth by their much lighter colour, and more delicate texture, may be found attacked by an aecidium-bearing mycelium. But, although, comparatively speaking, only a very few individuals of this widely distributed plant are thus attacked, yet when a plant is attacked, it is often largely so. Only these new young leaves, and sometimes the new stems bearing them, are attacked by the aecidium, and never the older leaves or stems. When the leaf blade is attacked, conspicuous bright yellow patches are formed, more or less irregular in shape, and varying in size from a small point to about 2 cm. in diameter (Fig. 1, Plate 1). These patches are, moreover, considerably thickened, and bulged, with their convexities usually towards the lower surface of the leaf. The short petioles are very frequently attacked, the young shoots rarely. A single leaf may bear from one to twelve patches of invasion, and if these be carefully examined when nearly mature, minute papillæ, brownish on their summits, are seen scattered over them both on the upper and lower surfaces, and these are the aecidia, which do not open widely, as in the other hitherto described Cæomata, but only by a pore through which the aecidospores are extruded in the form of small powdery masses. A still closer examination of these patches with a lens reveals some pellucid spots, especially on the upper surface, and these are spermogonia.

Later in the season, towards the end of September, and in October, the aecidal form begins to disappear, although a few aecidial patches may still, with diligent search, be found so late as the beginning of November. The leaves which bear aecidia apparently drop off early after the aecidiospores have been shed, for in November, although a very few ripe aecidial patches may be found, no dried-up patches can be discovered on still adherent leaves. But now, in October, a new phase of the fungus is revealed. On many leaves of the same generation which bore the aecidia, but which have now become tougher and darker green, a number of uredo-pustules may be found; and it is especially noteworthy that those leaves which still, at this late date, exceptionally bore aecidia, *always* presented many of these uredo-pustules, but at some little distance from the aecidial area, with intervening healthy tissue. In other words, the uredo-pustules are formed by a distinct mycelium with, as will be later noticed, very different characters and properties. This uredo-phase of the fungus life-history is also very much more widely distributed than the aecidial, and is to be found almost everywhere, wherever the host grows, and not only in certain restricted localities as in the case of the aecidial phase. At this time when the uredo stage is beginning to show itself, the lower surfaces of the leaves attacked exhibit a few, or a very great many, slightly paled circular areas

of mycelial invasion, on each of which appear minute pustules, containing yellowish brown uredospores. These invaded areas are not in the least thickened, and, as the slight paling of the green tissues caused by the mycelium is most pronounced on the lower surfaces of the leaves, where only the pustules are formed, the affection is by no means conspicuous, and in this respect offers a very striking contrast to the aecidial affection. In the special localities where the aecidium was formerly prevalent this uredo-affection is especially prevalent, and the attacked leaves present so many foci of attack that the circular areas of each separate attack frequently merge into neighbouring areas, and thus a large area of the leaf's under-surface is uniformly paled, and bespattered irregularly with uredo-pustules. But, when the leaves are not so extensively attacked, a very peculiar and characteristic arrangement of uredo-pustules is evolved. At first, after a circular paled area has been formed by the mycelium, a uredo-pustule usually breaks out from the centre of the area. A little later a circle of other pustules is formed around the central pustule. Later still, a second larger circle, around the first circle, is formed near the margin of the paled area. But before this last circle is formed the development of teleutospores, in the form of puccinia, has commenced in the earliest formed uredo-pustules, and especially in the first circlet of these. This teleutospore formation begins to take place in November. The originally very small uredo-pustules enlarge greatly when teleutospore formation sets in, so that the neighbouring pustules of the circlet often coalesce, sometimes wholly, when a complete circular wall of teleutospores is formed round the central pustule, which does not enlarge to any great extent, and which often does not form any teleutospores, until a much later date. More usually this circular wall of teleutospores is broken in continuity at one or two places. With the onset of the winter the continued production of teleutospores goes on slowly, but apparently more or less continuously, and in the following spring the condition of the attacked leaves is as follows. Such leaves now exhibit slightly paled circular patches, varying in number from one to about twenty, without any thickening or hypertrophy. The larger patches measure from 6 to 7 mm. in diameter and the smallest about 2 mm. The teleutospore beds are aggregated together circularly in hemispherical dark-brown compact masses (Fig. 2, Plate I). Sometimes in the smallest paled areas there is only one such bed of spores in the centre of the discoloured area, indicating the formation in the previous autumn of only one uredo-pustule, but usually there are two or more segments of a circle, around a central space, which contains the first formed uredo-pustule now containing a few teleutospores, with remains of uredospores. When a leaf has been extensively attacked the circular arrangement, as already explained is lost, but otherwise the circular teleutospore beds are surrounded by the second circle of uredo-pustules, about nine to ten in number. This outermost circle of pustules contains frequently only old uredospores, and perhaps a few

teleutospores, though sometimes these pustules also produce many teleutospores. They do not, however, enlarge and coalesce to form another ring.

In this condition the fungus awaits a fresh season of growth for the host at the onset of the rainy season, when the young leaves are again attacked by a mycelium bearing aecidia, thus completing the circle of development.

Before proceeding now to a description of the microscopic characters of the fungus, I may mention that if a leaf bearing teleutospore beds be placed in a moist chamber for some hours, the spore beds swell very noticeably, and become lighter brown in colour, or even brownish yellow. This swelling is due, as will be more particularly explained further on, to the swelling of a gelatinous sheath, enclosing the stalks of the teleutospores, as in the case of Gymnosporangium spores. The amount of swelling, however, in the case of this Smilax fungus is much less than it is in Gymnosporangium, not so much because the gelatinous sheaths themselves swell less, but because the stalks are very considerably shorter than those of Gymnosporangium spores. This peculiar structure of the stalks of the teleutospores is interesting in its resemblance, not only to Gymnosporangium spores, but also to the teleutospores of *Puccinia Berberidis*, Montagne, occurring on the leaves of *Berberis glauca*, found on the Island Juan Fernandez, and figured by De Bary in the plate illustrating his paper on *Aecidum abietinum* [1] I will recur to these resemblances later.

Microscopic characters of the Fungus.—The mycelium which bears the uredo and teleutospores calls for no special description. It does not give rise to any hypertrophy of the host's tissues, nor does it form haustoria. The hyphæ contain colourless translucent matter, and are therefore difficult to recognise (Fig. 6, Plate I).

The aecidium-bearing mycelium has, however, a very different character. It gives rise to very marked changes in the tissues of the host (Fig 4, Plate I), with which it comes into contact, and, as the hyphæ contain orange red globules in abundance, they are readily recognisable in fresh sections. Moreover, the mycelium gives rise to the accumulation of nutritive material in the parenchyma cells among which it ramifies. The amount of hypertrophy caused in the leaf tissue is considerable, making the thickness of the lamina three times its normal size when the parasite is fully developed. The effect upon the host's tissues produced by this fungus is clearly shown in figures 4 and 5, Plate I, representing sections of the normal and attacked leaves sketched under exactly similar conditions. In the first place it will be observed that in the normal leaf there is no division of the subepidermal tissue into palisade and spongy cells, all being of the latter kind. When the leaf is attacked by the aecidial parasite, these spongy cells are greatly hypertrophied, whilst mycelial filaments permeate the tissue, sometimes destroying the cells but never apparently entering them as haustoria (Fig. 7, Plate I). In the normal leaf it is also seen that the fibrovascular bundles usually extend through the entire depth of the lamina,

[1] Botanische Zeitung, 28th November 1879, No. 48, p. 846.

and these bundles are often seen to limit abruptly the extension of mycelial invasion. When the petiole is attacked it is also greatly hypertrophied, the areas of transverse sections through normal and attacked petioles being in the ratio of about 100 to 170. Such transverse sections, through attacked petioles, show that the aecidia are laid in the fundamental parenchyma tissue, below the hypoderma. The fundamental parenchyma cells are throughout considerably hypertrophied, for, whilst in transverse sections the normal cells measure in outline from $57 \times 38\mu$ to $82 \times 50\mu$, those among mycelium measure from $95 \times 63\mu$ to $139 \times 88\mu$.

The *teleutospores* by transmitted light are pale yellow, double-celled bodies, Puccinia, with long stalks, which, as already stated, are surrounded by a gelatinous sheath, swelling considerably in water (Fig. 13, Plate I). The free end of the upper cell is usually perceptibly thickened. The spores vary greatly in size from a total length of 74·0 to 50·8μ. The upper cell was found to vary in size from 38μ in length by 16μ in breadth to $25 \times 15\mu$, and the lower cell from $36 \times 16\mu$ to $25 \times 15\mu$. The spore is slightly constricted at the septum, which measures usually 14μ in breadth. These spores are detachable with difficulty, not only from their beds, but from one another, as they tend to break off in masses when scraped off by a needle. The stalks are from 2 to $2\frac{1}{2}$ times as long as the whole length of the spore, *i.e.* about 125 to 156μ, and when swelled in water they are about 12μ in diameter. A nuclear space is sometimes clearly defined in each cell, but not always. The epispore is smooth and without any markings. When the stalk is swelled in water the central thin axis is clearly defined, as in Gymnosporangium. The spores germinate in water very readily, throwing out a promycelium from each cell, into which the pale yellow contents of the spore body wander, accumulating in the distal extremities (Fig. 14, Plate II), leaving the proximal parts filled with colourless fluid. The promycelium of the upper cell emerges from the apex of the cell, and that of the lower from a point near the septum, as in most Puccinia, but unlike Gymnosporangium. From this point the further developmental phenomena are curious, and until the issue of the last Botanische Zeiting,[1] which I have just received, unlike those of any hitherto described species. The observations made in this number of the Botanische Zeitung by F. Kienitz-Gerloff on the peculiar development, *at times*, of the teleutospores of *Gymnosporangium clavariæforme* add considerable interest to the Smilax fungus, for, whilst before there was only a morphological resemblance, which might well have been of little or no importance, we have now a biological resemblance, and both together suggest a closer relationship between Gymnosporangium and the other Æcidiomycetes than has hitherto been suspected. The ends of the promycelia certainly usually divide into four cells (Fig. 15*c*, Plate II) by transverse septa much in the usual way, but those cells, instead of producing sporidia, at the ends of sterigmata, round off at

[1] Botanische Zeitung, No. 25, 22nd June 1888, p. 389.

the septa, and separate, and apparently represent, sporidia. Frequently only three of the four cells formed thus separate, the fourth not maturing. These detached cells, or sporidia, have usually one or two vacuolated spaces, and measure from $14 \times 8\mu$ to $18 \times 11\mu$ (Fig. 15 *a*, *b*, *c*, Plate II). I have never, however, succeeded in getting them to germinate, unless, indeed, Fig. 15 *a*, plate II, represents the germination of a spore not yet detached; but, on the other hand, this may, and I think probably does, represent an abortive attempt at the usual sporidial formation through a sterigma. Of the numerous cultivations I examined carefully this was the only case exhibiting such growth, all the rest developing exactly as I have described and depicted. The absence of any observed germination of these sporidia makes one serious gap in the life-history of this fungus. It may also be urged that this peculiar sporidial development is due to the unsuitable media (water and a decoction of Smilax leaves) in which I placed the teleutospores. This of course is possible, but against this view I may note that it, and one other teleutospore, to which I shall presently allude, are the only ones of the many teleutospores I have observed germinating, which develope so, and that they *invariably* develope so most readily, and without any sign of hesitation. In 24 hours a cultivation presents innumerable such sporidia. The single other case to which I have alluded, is a Uromyces on *Solidago verga-aurea*, Lin., which behaves in exactly the same way, and in which, moreover, the peculiarly developed sporidia germinate readily in water. Figure 16, Plate II, represents the teleutospore before germination; Figure 17 *a*, one which has commenced to germinate, but in which the promycelium still remains undivided; and Figure 17 *b*, the cast-off terminal end of another promycelium, with four sporidia still adherent to one another, formed just as those of the Smilax fungus are, in two of which germ tubes are thrown out. I will not here encumber this paper with a detailed description of this Uromyces, but merely mention those points in support of the views I take, that this sporidial formation is a new type of normal germination.

The *uredospores* are oval, or more usually pyriform, pale yellow bodies, beset externally with very prominent spines (Fig. 12, Plate II), and so closely resemble the aecidiospores as to make it difficult to distinguish the one from the other. Figure 9, Plate II, represents some spores from leaves which were collected early in October, and preserved in botanical drying paper. The spores were sketched late in November after the leaves had been 24 hours in a moist chamber. These may be compared with fig. 8, Plate II, representing aecidiospores drawn under exactly similar conditions. Among the uredospores a few club-shaped paraphyses may always be seen. The spores are formed singly on short stalks about the length of their own long diameter (Fig. 6, Plate I). The fresh spores, after lying a few minutes in water, measure on an average $46{\cdot}5 \times 31{\cdot}7\mu$. The epispore is thickened at the broad free end. These spores do not germinate at all readily in water: only in one of many cultivations did a few germinate, throwing

out a usual, long tube, sometimes with short branches. This cultivation, made on the 11th October, contained new matured spores.

The *aecidium* is deeply placed both in the leaf and in the petiole, and is not bounded by any peridium, but by a layer of convoluted hyphæ (Fig. 4, Plate I). It opens by a narrow mouth both on the upper and lower surfaces of the leaf. The hymenium is curved and the aecidiospores are given off successively from basidia, but the ripe spores do not remain attached to one another, forming rows, as usual. On the contrary, as each spore ripens, it is cast off, and the cell below, which up to this time remained in a rudimentary condition, then grows rapidly, forming another ripe spore, and so on (Figs. 18 and 19, Plate II). The spores are pale yellow, mostly oval, with an epispore of variable thickness, thickened at one end, and beset with large coarse spines, which are deciduous. When just wetted, the spores measured on an average 43·2 × 25·6μ, varying from 36 × 28μ to 52 × 16μ. The thickness of the epispore is usually about 4μ and 6 to 10μ at the thickened end. These spores, like the uredospores, do not germinate at all readily in water. In one only of the numerous cultivations I made had a few commenced to germinate after four days. Some of these had thrown out two small germ tubes (Fig. 11, Plate II), and some only a single one (Fig. 10, Plate II). Beyond this they never went, the germ tubes were 8μ in diameter.

The *spermogonia* occur plentifully both on the upper and lower surfaces of the leaf, but more especially on the upper. They are deeply sunk below the epidermis (Fig. 3, Plate I), and through their mouths a tuft of brittle paraphyses protrude. They are of the usual structure, and measure when ripe, 145μ in depth and 157μ in breadth, the paraphyses protruding about 50μ beyond the mouths. Even in this stage, and before the inception of aecidia, the leaf tissues are greatly hypertrophied, one measurement made showing the depth of the laminal tissue in invaded areas to be 504μ against a normal depth of 296μ close by.

Concluding Remarks.—This completes the description of the fungus, and it now remains to add a few remarks on its life-history, and the affinities suggested with other Uredineæ.

I made many attempts to reproduce the aecidial form by sowing teleutospores on the young newly developed leaves at the time when the aecidial form occurs in nature, but, with one exception, these attempts failed. I do not consider the one success sufficient in itself to establish the genetic relationship, as the possibility of accidental, and unintentional, infection must, in such case, be admitted, but taken with the natural history of the fungus, the one success makes it extremely probable that we have here an autœcious uredine. I am inclined to attribute the negative results of all my other experiments to the absence of some necessary external condition, which I have not yet been able to discover, and not to the teleutosporic form being foreign to the aecidial; and I am supported in this view on two grounds, first, because I sowed the same teleutospores, during three years, on several other hosts which bore other aecidia in the neighbourhood, and, *vice versâ,* other teleutospores from other hosts

on Smilax without any result, and secondly, because even in nature, although the teleutospores are widely and plentifully distributed over the Simla region, the aecidial form is found only in certain few localities, where only, I presume, the special conditions necessary for attack exist (these localities were moist, densely wooded, and therefore very shaded). The resemblance of the teleutospores to those of Gymnosporangium induced me also to make many attempts to reproduce an Aecidium (Roestelia) on *Pyrus variolosa*, Wall., which in certain years is very common in Simla, but without success. This Roestelia is the only representative of the genus in Simla, and there can, I think, be no doubt that it is caused by the only Gymnosporangium in Simla, one on *Cupressus torulosa*, Don. I have, however, elsewhere[1] shown that, although these last two fungi must be related, I could never demonstrate it by experiment. And this case shows very forcibly how easily inoculation experiments may fail, because it is scarcely conceivable that this only Gymnosporangium, which occurs in Simla, is not related to the only Roestelia we have. There is also this parallelism between the Pyrus and Smilax Aecidia that both are relatively rare in comparison with the largely distributed, and readily germinating teleutospores, and here, too, I think my inoculations have not succeeded, because some special unknown condition, comparatively rare even in nature, must be present before attack by the teleutospores can take place.

I was even more unsuccessful in attempting to produce the uredo-form by sowing with aecidiospores, for all my experiments in this direction were fruitless. The fact, however, that in nature, in October, the leaves which bore aecidia were also invariably attacked by the uredo-producing mycelium lends strong support to the view that this was the result of infection by the aecidiospores produced on the same leaf.

The total result therefore is that, although unimpeachable evidence does not exist to connect the two forms of fungus with one another, such a connection is rendered in the highest degree probable by a consideration of the facts above set forth.

In my introductory remarks I noted that I felt doubtful whether the phase I have described as aecidial were really so or not. I doubted because (1) the aecidia were unlike other aecidia in opening by a pore, (2) because the aecidiospores were not formed in rows as usual, and (3) because the aecidiospores are morphologically scarcely distinguishable from the uredospores. But, as I have said, I have now no longer any hesitation on this point, because the character and properties of the mycelium producing the aecidia are very different from those of the uredo-producing mycelium, and because the acidia are always accompanied by spermogonia, and the mycelium which produces these organs never produces teleutospores. The reason why the aecidiospores are not formed in rows may, I think, be due to the aecidia not opening widely enough to admit of this. As soon as a few aecidiospores are formed within the limited capacity of the aecidium there is no room for others until some are extruded.

[1] Journal of the Asiatic Society of Bengal, Vol. LVI, Part II, No. 3, 1887.

Lastly, a few remarks with reference to new affinities suggested by the new type of sporidial formation displayed. Had it not been for Prof. R. Hartig's discovery of the life-history of *Cæoma pinitorquum*, in connection with *Melampsora populina*, the only other Cæoma, I believe, whose life-history is known, I should naturally have been inclined to regard this new type of sporidial formation as probably peculiar to Cæoma. In the Simla region we have, so far as I know, only one Cæoma other than that on Smilax, namely, on the leaves of *Morus alba*, Lin., var. *serrata*, and it is possible that the Uromyces I have alluded to on *Solidago verga-aurea*, L., presenting the same type of sporidial formation, may be connected with it. However, whether this be so or not, Hartig's discovery shows that it is not a necessary characteristic of the Cæomata.

Kienitz-Gerloff thinks that the somewhat similarly produced spores from the promycelia of *Gymnosporangium clavariæforme* are in nature uredospores, but I do not think this view will be readily accepted. Looking at the three forms, (1) *Puccinia Berberidis*, Montague, (2) *Gymnosp. clavariæforme*, and (3) *Cæoma Smilacinis* and the Uromyces on *Solidago verga-aurea*, it appears to be more probable that we have here an evolution series, but in which direction, whether from (1) to (3) or from (3) to (1) there are not sufficient data to determine. As the process of sporidial formation is simpler in *Cæoma Smilacinis*, as I propose to call this fungus, and in the Uromyces on *Solidago verga-aurea* than in other cases, these may probably represent the earliest stages in development towards the usual mode. It is conceivable that the earliest teleutospores were hardier uredospores, which tided the fungus over unfavourable seasons, and then germinated as usual, throwing out a germ tube which directly penetrated the host (autœcious). From this might be evolved a form like *Cæoma Smilacinis*, and through this the usual form with sporidia at the ends of sterigmata. In such case *Gymnosporangium clavariæforme* would represent a form still retaining traces of a former stage of development.

The 14th July 1888.

Description of the Plates.

PLATE I.—1. *a* lower and *b* upper surface of leaf attacked by æcidial form; natural size.

2. Lower surface of leaf gathered in spring with uredo and teleutospore-pustules, showing circular arrangement; natural size.

3. Transverse section of leaf through spermogonium × 350.

4. " through æcidium, × 150. Fig. 5 is drawn from the same section at an unattacked part.

5. Transverse section of normal leaf, × 150.

6. " leaf through uredo-bed, × 150

PLATE II.—7. Portion of transverse section of leaf through an æcidial patch, showing mycelium between sporiferous cells, × 350.

8. Group of æcidiospores from dried specimen, moistened in water, × 350.

9. Group of uredospores, also from dried herbarium specimen, moistened, in outline only, × 350.

10. Æcidiospore showing commencing germination; only one germ tube, × 350.

11. Æcidiospore showing two germ tubes, × 350.

12. Fresh uredospore, in water. × 350.

13. Teleutospore in outline, showing young, still undivided promycelia from each cell, × 300.

14. Upper ends of two promycelia from same spore just before division, × 350.

15. The same from another spore: *a*, three sporidia have been formed, one of which has thrown out a germ-tube (or abnormally a sterig ma?); *b*, two sporidia are ripe and ready to be cast off; *c*, one sporidium ripe and about to be cast off, while two others are still immature, × 350.

16. Uromyces (teleutospore) from leaf of Solidago Virga-aurea, × 350.

17. " ; *a*, showing promycelium still undivided; *b*, showing upper end of another promycelium consisting of four cast-off sporidia, still however attached to one another, two of which have thrown out germ tubes × 350.

18. Section showing portion of æcidium to illustrate the manner in which æcidiospores are formed, × 350.

19. Portions from other similar sections to illustrate more clearly the same thing, × 350.

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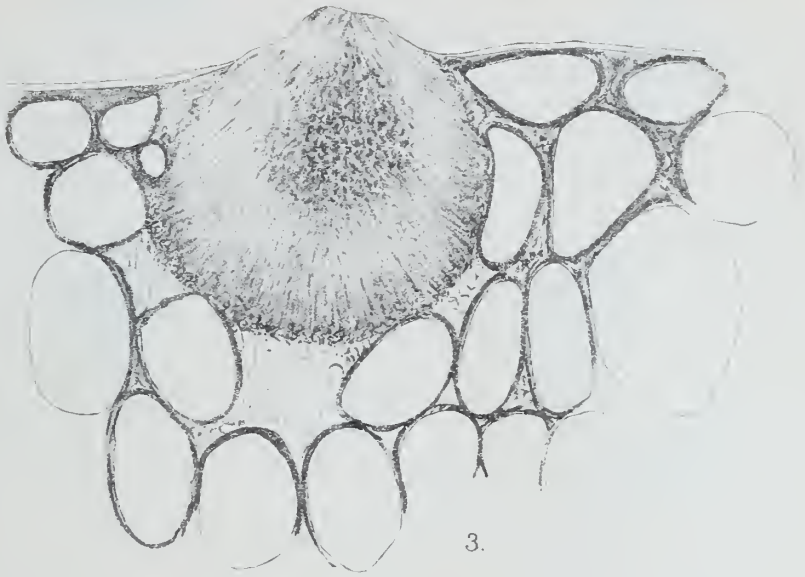

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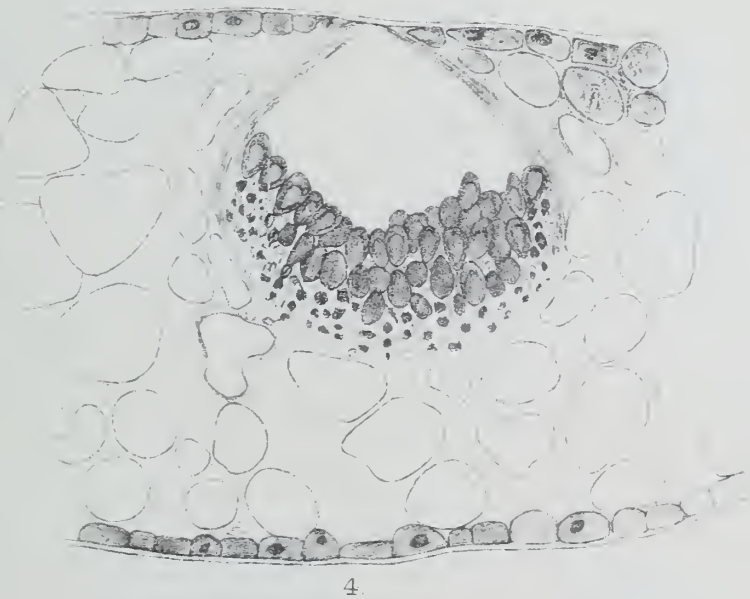

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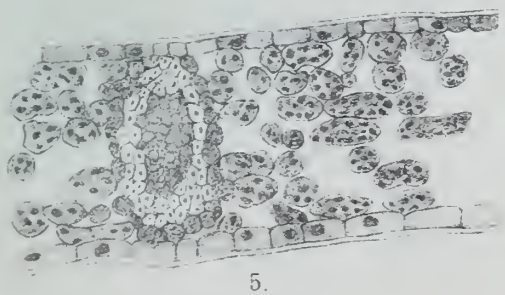

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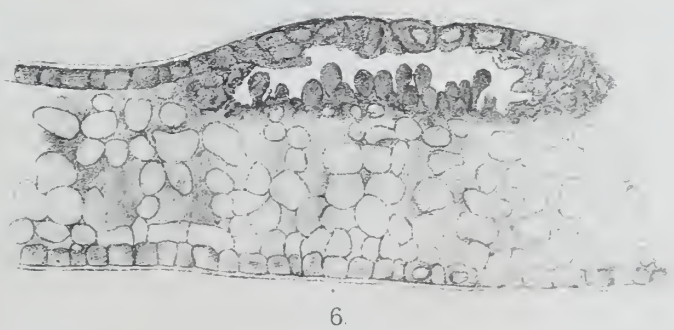

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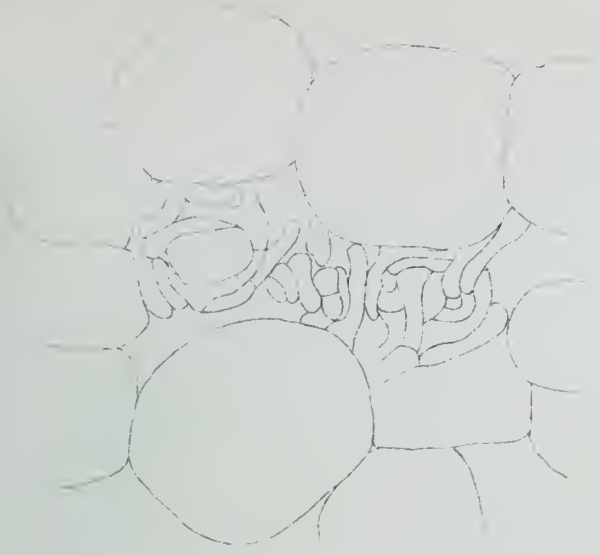
A. Barclay, del.

CAEOMA SMILACINIS.

Lithographed at the Survey of India Offices, Calcutta, September 1868.



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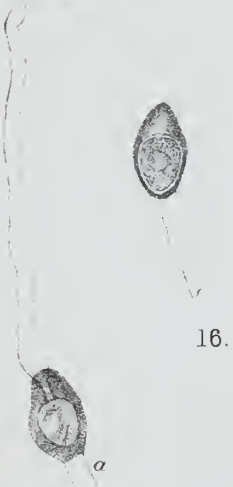

16.

a.

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a.

19.

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A. Barclay, del.

CAEOMA SMILACINIS.

Lithographed at the Survey of India Offices, Calcutta, September 1888.

Are Venomous Snakes auto-toxic?

An Inquiry into the Effect of Serpent-Venom upon the Serpents themselves.

BY

Surgeon L. A. WADDELL, M.B.,

Deputy Sanitary Commissioner, Bengal.

INTRODUCTORY.

Practical bearing of inquiry.

An interesting question which presents itself in studying the phenomena of serpent-poisoning is that which refers to the insusceptibility or otherwise of a serpent to its own venom, or that of its fellows. And this question is not without a practical bearing upon the treatment of snake-bite: for, were such immunity proved to exist, a study of its conditions might possibly afford indications for combating the action of the venom on man.

Seeming anomaly of subject.

That an animal should be subject to poisoning by one of its own normal secretions, must, *primâ facie*, seem improbable, as this would prove detrimental to the individual and to the species. And especially so, when, as in the present case, the exposure to poisoning would be habitual: for snakes are frequently receiving accidental injuries to the mouth, with abrasion of the mucous membrane of the buccal cavity, and thus, not unfrequently, must absorb some of their own venom.[1]

Should, on the contrary, snakes be proof against poisoning by their own venom, then the problem presents itself of how to account for the organism of the serpent being able to resist a chemical poison which is so deadly to most other animals.

As to whether such insusceptibility really exists, authorities differ widely in opinion.

Conflict of opinion on question.

On the one hand are those who assert the existence of this insusceptibility:—

Favouring view of insusceptibility.

Fontana, in 1765, experimenting on the European Viper (*Viper aspis?*) concluded that "the venom of the viper is neither a poison to the viper itself, nor to those of its own species."[2]

[1] This fact was pointed out long ago by Fontana in regard to the Viper ('*Treatise on the Venom of the Viper, &c.*,' Skinner's transl. London, 1795, Vol. I, p. 274).

[2] *loc. cit.* p. 34. His experiments on this head, over twenty in number, were admirably conceived and carried out.

Russell,[1] Fayrer,[2] Richards,[3] Nicholson,[4] and Hopley [5] give results of experiments or observations which led them to conclude that the cobra (*Naja tripudians*) is insensible to its own venom or to that of its fellow species. And Indian snake-charmers also have come, by experience or tradition, to hold the same belief.

Breton [6] and Fayrer [7] extended to poisonous snakes in general this principle of insusceptibility.

And analogous experiments upon scorpions, by Bourne [8] and others [9] led to the conclusion that the poison of a scorpion is quite powerless to kill the same individual, or another individual of the same, or even of another species.

In favour of susceptibility.

On the other hand, Professor Weir Mitchell of Philadelphia—whose very elaborate researches entitle him to the first rank as an authority on snake-venom—states [10] that he felt "at liberty to conclude that the animals (*crotali*) . . really died from the venom (*crotalus*)." And [11] that the venom of the rattlesnake is "poisonous . . to its owner" "as well as to other animals." Mitchell's experiments in this direction were confined to rattlesnakes, but it is unlikely that the principle involved should differ materially in the case of the rattlesnake from that of the cobra and other venomous snakes. Popular reports also credit venomous snakes occasionally with biting themselves with rapidly fatal results.[12]

Mixed views.

As intermediate between these antagonistic views may be cited the apparently contradictory opinions of Fayrer and of Richards. Although Fayrer, in summarizing his con-

[1] "*An Account of Indian Serpents*," Lond. 1796, p. 56. He made one cobra bite another, with the result that the bitten cobra "remained well." "This experiment was repeated with the like result." This cobra some days afterwards was bitten in the belly by another cobra, "blood appeared on the wound but no other consequence followed." While an innocent snake, "a tartutta (*Dipsas trigonata*?), bitten immediately after in the same, part, died within two hours."

[2] "*The Thanatophidia of India*," Lond. 1872, p. 66 *et. seq.*

[3] *Idem*, p. 125 *et seq.*

[4] "*Indian Snakes*," 2nd Ed., Madras, 1874, p. 145.

[5] "*Snakes: Curiosities and Wonders of Serpent Life.*" Lond. 1882, p. 563.

[6] *Trans. Med. and Phys. Soc.* Calcutta, 1826, Vol. I, p. 170. This conclusion, however, was based upon one solitary experiment on a cobra and a *Daboia russellii* biting each other.

[7] *loc. cit.* p. 64, "The poisonous snakes are not affected by their own poison." In support of this statement is given, in addition to the experiments on cobras, one case of a 'krait' (*Bungarus cæruleus*) bitten by another krait with "no effect" (p. 134); and Richards reports (p. 127, *idem*) another case in which the small bitten krait was "found dead" the following morning. But no further experiments seem to have been made upon kraits, and none upon any of the other species of venomous snakes of India. Regarding the effect of venom upon another venomous snake, Fayrer states (p. 73) that the experiment recorded "seems to prove that the venomous snakes have no power of poisoning each other," and (at p. 64) "in many of the various experiments I have performed, the Cobra, *Daboia* and Krait did not appear able to poison themselves or *each other*."

[8] *Proc. Roy. Soc.*, p. 20 xlii, 1887. He experimented on three species of scorpions found in Madras, with the view of determining whether scorpions can commit suicide.

[9] Ray Lankester and others, from experiments made at Cape Town, about six years ago, arrived, I am informed, at somewhat similar conclusions; but I can find no published record of their results.

[10] "*Researches on the Physiology and Toxicology of the Venom of the Rattlesnake.*" (Vol. XII, Smithson. Contrib.) Washington, 1860, p. 63.

[11] *Idem*, p. 43.

[12] *Nature*, Vol. XXII, p. 40. And Fontana and Mitchell refer to the currency of this belief.

clusions states at one place definitely and unreservedly,[1] that "the poisonous snakes are not affected by their own poison," yet, he notes on the following page that, although "in many of the various experiments I have performed, the Cobra, Daboia, and Krait did not appear able to poison themselves or each other, *some of the experiments render this doubtful.*"

And Richards, although noting[2] that "I believe one cobra cannot poison another" and[3] "I am quite satisfied the cobra cannot kill another cobra;" and recently[4] he relates how healthy cobras, which he kept together in captivity, would "very often on the slightest provocation begin to fight in a most savage" fashion, biting each other fiercely, with the result that "neither of the combatants ever seemed any the worse for the fight." Yet, he notes,[5] "I came to the conclusion, after numerous experiments, that one species of snake could kill another" (by the context) venomous snake of the same species.[6]

Such conflict of opinion upon so elementary a point rendered a further investigation of the subject desirable—especially, as the methods adopted by several of the foregoing experimenters, to elucidate the point at issue, were open to objection.

Desirability of further investigation.

Part I.—Working Scheme adopted.

The inquiry resolves itself into a consideration of the effect of venom (1) upon the serpent itself and its own species, (2) upon venomous snakes of other species, (3) upon innocent snakes, (4) upon other reptiles and cold-blooded animals, (5) upon warm-blooded animals; and of the topics arising out of the results of the above investigations.

Scope of inquiry defined.

It is claimed for the present series of experiments that they have avoided many of the sources of experimental error to which those of former observers were liable:—

Sources of experimental error.

The method usually employed to ascertain the toxic effect of snake-venom upon snakes had been to force one snake to bite another, or its own tail. In this way, however, there is always a probability that the spine or viscera of the bitten snake were crushed, or otherwise grossly injured during the act of biting.[7]

Injury to viscera, &c., during infliction of bite.

Again, in employing biting as a means of introducing venom, there is uncertainty as to the amount of venom introduced; and in some cases uncertainty as to whether any venom at all has been injected—owing to the want of anatomical continuity

Uncertain introduction of venom by biting.

[1] *loc. cit.*, p. 64. [2] *idem*, p. 126. [3] *idem*, p. 127.

[4] *Landmarks of Snake-poison Literature.* 2nd Ed. Calcutta, 1886, p. 12.

[5] *idem*, p. 12. [6] No details of the experiments are given.

[7] In Fayrer's series of cobras-bitten-by-cobras no *post-mortem* examination appears to have been made to ascertain whether any such fatal injury had been inflicted.

between the venom-duct and the canal of the fang and the more or less erectile nature of the fang—especially in vipers.[1] The "difficulty in making the viper (*Daboia*) insert its long slender fangs into the tough skin of the cobra' is noted.[2] The venom may in part be washed out with the blood escaping from the wounded part.[3] The snake may have recently shed its venom and have little more available.[4] Snakes are also credited with being able to control the flow of their venom, expelling a larger quantity when irritated and rendered furious than in ordinary biting.[5]

Mortality of captive snakes.

A generally recognised difficulty in interpreting the results of such experiments is the frequent mortality of captive snakes. In Fayrer's experiments the majority of the snakes operated upon may have been fresh, but the fact is only noted in regard to one of the bitten snakes. Mitchell's experiments appear to have been made upon snakes which had been in confinement for a considerable time.[6]

Necessity for prolonged observation.

The necessity for prolonged observation of the envenomed snake is strongly insisted upon by Mitchell[7] and Claude Bernard[8] on the plea that cold-blooded animals are very much less rapidly affected by venom than the warm-blooded, and hence the necessity, in their opinion, for the snake being kept under observation during a period of several consecutive days.[9]

Using venom of unascertained activity.

It is also desirable that the venom experimented with be of ascertained activity. This precaution had been very seldom observed.

General precautions taken.

In the present series of experiments the above-noted sources of error were to a considerable extent avoided or minimized by the employment of freshly-caught uninjured snakes, by introducing without loss a measured quantity of the venom by the more precise mode of hypodermic injection,[10] by prolonged observation, and by resorting to numerous control experiments to test the activity of the venom.

[1] Dr. MITCHELL notes (*loc. cit.*, p. 25), "I have seen the Rattlesnake strike with great apparent ferocity a number of times, when I have been unable to discover any fang wound whatsoever." Again, "in some cases it is quite possible that the relations of the fang and the duct are so disturbed that the venom never enters the tooth at all." And "It sometimes happens that the blow is given, the fang enters, and from the quick starting of the animal injured, or from some other interrupting cause, it is withdrawn so soon that the larger portion of the poison is thrown harmless upon the surface near the wound."

[2] FAYRER, *loc. cit.*, p. 92. [3] FONTANA, *loc. cit.*, p. 139. [4] *idem*, p. 140.

[5] *idem*, p. 139. [6] *loc. cit.*, p. 61. [7] *loc. cit.*, p. 63.

[8] "*Leçons sur les Effets des Substances Toxiques, etc.*" 1857, p. 291—quoted by Mitchell, who affords a very full bibliography of serpent-venom literature.

[9] In a fourth of Fayrer's experiments the observations were continued for only one day, and in another fourth for two days only.

[10] FAYRER, *loc. cit.*, p. 75 *et seq.*, records seven cases in which cobra-venom was introduced hypodermically into cobras, but in only two was the observation extended till the sixth day, and in none beyond the sixth day, and no control observations were made.

Part II.—Effect of Venom upon the Serpent itself, or its own Species.

The Experiments.

The experiments under this head were confined to the cobra (*Vaja tripudians*), as, with the exception of tree-vipers, other species of venomous snakes were not available. And the experiments, with one exception, dealt with the strictly auto-toxic aspect of the subject. Concerning the ability of one venomous snake to poison another individual of the same species, the one experiment (No. XX) here afforded seems almost sufficient when taken in connexion with the numerous experiments recorded by Fayrer[1] and others,[2] in which one cobra was forced to bite another cobra. Moreover, snake-venom (*e.g.*, cobra or *crotalus* venom), from whatever individual derived, is found to possess for each species such constant and well defined properties, that it may well be regarded as a specific chemical poison;[3] and as such it is highly probable that the venom shall act upon another snake of the same species in an identical manner to its action upon the owner of the venom.

General arrangements.

The number of cobras operated on was nine, in three series of four, four, and one respectively. I had hoped to operate on a larger number, but failed to procure more by my unaided private efforts within the limited time at my disposal.

The first series of experiments was conducted at the end of August of the current year, the temperature of the room in which the snakes were kept, ranging from 81° to 86° Fah. The second series was conducted at the beginning of October, the temperature ranging between 65° and 88° Fah. And the third series at the beginning of November, the temperature ranging between 54° and 78° Fah.

The serpents operated on were all healthy, and had been caught within the previous two to seven days.

In the experiments the venom was extracted in the usual Indian way by causing each cobra to bite through a strip of dried palm leaf[4] stretched across a spoon or valve of a mussel-shell. For injecting the venom an ordinary hypodermic syringe was used. And during the process of injection the serpent was secured by a noose around the neck after the manner recommended by Mitchell.

Details of the Experiments.

First Series of Cobras.

EXPERIMENT I.—A binocellated cobra (*'Gokhura'*), measuring 53 inches in length, was made to disgorge its venom. About 18 minims of clear venom were

[1] The results of these scattered experiments are collected and analyzed at p. 57 (*q. v.*)

[2] RUSSELL and RICHARDS, *loc. cit.*

[3] For evidence on this head *vide* Part VI.

[4] *Borassus flabelliformis.*

obtained. This venom was mixed with an equal bulk of water, and at 9-15 A.M. 25 minims of this solution were injected hypodermically (and very slightly supra-muscularly) about the middle of the back of this cobra—the same which had yielded the venom. Atmospheric temperature 82° Fah.

This snake was observed every hour during the day and seemed unaffected.

Next day.—	Active and well.	Fierce when disturbed.
3rd	„	Ditto.	
4th	„	Ditto.	
5th	„	Ditto.	Taken out of its cage it bit eagerly, yielding about 9 minims of clear venom.
6th	„	Ditto.	
7th	„	Ditto.	
8th	„	Ditto.	
9th	„	Ditto.	Taken out of its cage it bit vigorously, yielding about 4 minims of slightly cloudy venom. It was immediately thereafter killed and dissected.

Post-mortem Examination.—Wound healed, and at site of injection no staining or softening of tissues. All organs strictly normal in appearance.

To test the activity of the venom employed in Experiment I the following two experiments were made immediately after injecting the cobra :—

Experiment II.—The remainder of the same venom solution as was used in Experiment I was diluted with an equal volume of water, and of this solution 6 minims were injected hypodermically into inner side of thigh of a chicken weighing 14½ ounces. Atmospheric temperature 82° Fah.

9-19 A.M.—Time of injection.
9-22 „ —Unable to stand, beak resting on ground.
9-23 „ —Convulsions ; passing fæces.
9-30 „ —Almost dead.
9-33 „ —Dead.

Experiment III.—Nine minims of same solution as used in last Experiment (II) were injected hypodermically into thigh of chicken weighing 15 ozs. Atmospheric temperature 82° Fah.

9-24 A.M.—Time of injection.
9-28 „ —Drooping.
9-30 „ —Convulsions.
9-35 „ —Almost dead.
9-41 „ —Dead.

Experiment IV.—A very fierce monocellated cobra (*'Shánkha mutiyá Ki-otiá'*) measuring 53½ inches in length, was made to disgorge its venom—yielding about 20 minims. This venom was diluted with an equal bulk of water, and at 10-5 A.M., 25 minims were injected hypodermically about middle of back of this same cobra which had yielded the venom.

This snake was observed every hour during the day and seemed to be unaffected.

Next day.—Active and well. Very fierce when disturbed.

3rd to 9th day.—Active and well. Very fierce when disturbed. It was taken out of its cage on the fifth and ninth days and bit eagerly, yielding clear venom. On the ninth day it was killed and dissected.

Post-mortem Examination.—The skin over site of injection slightly dry and wrinkled, but no subjacent exudation or softening of tissues—only a faint capillary staining subcutaneously at seat of injection. Organs strictly normal in appearance.

To test the activity of the venom used in Experiment IV the following two experiments were made:—

EXPERIMENT V.—Of the remainder of the solution used in Experiment IV, 8 minims were injected subcutaneously into thigh of a chicken weighing 14 ozs.

10-18 A.M.—Time of injection.
10-20 ,, —Prostrate; beak resting on ground.
10-25 ,, —Convulsions.
10-28 ,, —Dead.

EXPERIMENT VI.—Of same solution as used in last experiment 4 minims were injected into thigh of a chicken weighing 10 ozs.—

10-21 A.M.—Time of injection.
10-28 ,, —Convulsions.
10-32 ,, —Dead.

EXPERIMENT VII.—A monocellated cobra ('*Adyat ki-otiá*'), measuring 43 inches in length, yielded about 12 minims of venom. This was diluted with an equal volume of water, and at 10-45 A.M., 12 minims were injected hypodermically into this same cobra, as in Experiments I and IV.

During the day no effect was observable, and the snake remained well and active up till the ninth day, when it was killed and dissected.

Post-mortem Examination.—Wound healed, no softening of tissues or exudation, very slight capillary staining limited to track of syringe-needle. Organs strictly normal.

To test the activity of the venom used in Experiment VII the following two experiments were made:—

EXPERIMENT VIII.—Of the remainder of the solution used in last experiment No. VII, 8 minims were injected subcutaneously into thigh of a chicken weighing 14 ozs.—

10-51 A.M.—Time of injection.
11-10 ,, —Convulsions.
11-23 ,, —Dead.

EXPERIMENT IX.—Of the same solution as was used in last experiment 4 minims were similarly injected into a chicken weighing 13 ozs.—

10-53 A.M.—Time of injection.
11-11 „ —Convulsions.
11-30 „ —Dead.

EXPERIMENT X.—A small binocellated cobra (' *Kálá Gokhura* '), measuring 34 inches in length, yielded about 10 minims of venom. This was mixed with double its bulk of water, and 15 minims of this solution injected into this same cobra. The injection appeared to be without effect. The snake was observed several times during that day and following days, and found at all times to be actively on the alert.

On the fifth and ninth days following injection, it bit eagerly, yielding clear venom. On the ninth day it was killed and dissected.

Post-mortem Examination.—Wound healed, no exudation, softening, of staining of the tissues at seat of injection. Organs strictly normal.

To test the activity of the venom used in Experiment X, the following two experiments were made immediately thereafter :—

EXPERIMENT XI.—Of the remainder of the solution used in Experiment X, 8 minims were hypodermically injected into a chicken weighing 14 ozs.—

11-28 A.M.—Time of injection into thigh of chicken.
11-37 „ —Convulsions.
11-42 „ —Dead.

EXPERIMENT XII.—Of the same solution as was used in last experiment 4 minims were similarly injected into a chicken weighing 10 ozs.—

11-30 A.M.—Injection into chicken's thigh.
11-36 „ —Convulsions.
11-43 „ —Dead.

Second Series of Cobras.

EXPERIMENT XIII.—A very large binocellated cobra, measuring 59½ inches in length, yielded about 9 minims of straw-coloured clear venom. This was mixed with an equal quantity of water, and of this solution 15 minims were injected hypodermically about middle of back.

During that day the snake appeared unaffected, and no change was noted during the subsequent fourteen days. On the fifteenth day after injection, the snake was active and furious, it bit vigorously, yielding a small amount of clear venom—the greater portion escaping into snake's mouth. It was immediately thereafter killed and dissected. Temperature during its captivity ranged between 65° and 78° Fah.

Post-mortem Examination.—No morbid appearances externally over site of injection. Subcutaneously slight limited swelling about size of small split-pea

beneath fascia, which had been pricked by injection needle, but no softening or exudation. Viscera strictly normal in appearance.

To test the activity of the venom used in Experiment XIII, the following experiment was made:—

EXPERIMENT XIV.—The remaining 3 minims of the solution used in experiment XIII were injected hypodermically into inner aspect of right thigh of a chicken weighing 15 ozs.—

11- 9 A.M.—Time of injection.
11-17 „ —Head drooping.
11-20 „ —Lying on side paralysed.
11-22 „ —Convulsions.
11-33 „ —Nearly dead.
11-35 „ —Dead.

EXPERIMENT XV.—A large binocellated cobra (*'Kálá Gokhura'*), measuring $47\frac{3}{4}$ inches in length, yielded about 12 minims of light straw-colored clear venom. This was mixed with an equal bulk of water, and of this solution 20 minims were injected hypodermically about middle of back of this same snake. Temperature as in Experiment XIII.

During that day no change was apparent. Nor was any change evident up to the fifteenth day after injection, when the snake was taken out of its cage, and it bit fiercely, yielding clear venom. It was immediately thereafter killed and dissected.

Post-mortem Examination.—Slight wrinkling and dulness of skin over site of injection. No exudation or staining or softening subcutaneously; but point of needle had pricked the sheath of the spinal muscles, and sub-fascially around site of puncture was a small inflammatory nodule about size of a small pea, red on section, and of cheesy consistence. Viscera strictly normal in appearance.

To test the activity of the venom used in Experiment XV, the following experiment was made:—

EXPERIMENT XVI.—The remaining 4 minims of the solution used in Experiment XV were injected hypodermically into thigh of a fowl weighing 24 ozs.—

11-39 A.M.—Time of injection.
11-46 A.M.—Beak resting on ground.
11-53 A.M.—Lying on side with convulsive gaspings.
11-59 A.M.—Dead.

EXPERIMENT XVII.—A vigorous monocellated cobra (*'Shankha mutiya Ki-otiá'*), measuring $46\frac{3}{4}$ inches in length, yielded about 15 minims of clear colorless venom. This was mixed with an equal bulk of water, and 25 minims of this solution were injected hypodermically about middle of back of this cobra.

Temperature as in Experiment XIII. During that day it seemed unaffected. Nor did it seem affected during the fourteen subsequent days. On the fifteenth day after injection it was taken out of its cage and it bit fiercely, yielding clear venom. It was immediately thereafter killed and dissected.

Post-mortem Examination.—No local signs whatsoever. Organs strictly normal in appearance.

To test the activity of the venom used in Experiment XVII, the following experiment was made :—

EXPERIMENT XVIII.—The remaining 5 minims of the same solution used in Experiment XVII were injected hypodermically into the thigh of a fowl weighing 30 ozs.—

12 noon.—Time of injection.
12-4 P.M.—Sitting with beak resting on ground.
12-20 „ —Convulsions.
12-23 „ —Dead.

EXPERIMENT XIX.—A large binocellated cobra, measuring $51\frac{3}{4}$ inches in length, had 7 grains of cobra venom (obtained from another cobra one month previously and of ascertained activity[1]) dissolved in 15 minims of water injected hypodermically about middle of back. Temperature as in Experiment XIII.

During that day it seemed to remain unaffected, and so it also remained during the following fourteen days. On the fifteenth day following the injection, the snake was taken out of its cage, and bit vigorously, yielding clear venom. It was immediately thereafter killed and dissected.

Post-mortem Examination.—No local signs evident. Viscera generally were slightly congested, but not otherwise abnormal in appearance, and no fluid extravasation had occurred into any of the cavities.

Third Series of Cobras.

EXPERIMENT XX.—A large active binocellated cobra, measuring 58 inches in length, yielded about 16 minims of clear venom. This was diluted with an equal bulk of water, and of this solution 28 minims were injected hypodermically about middle of back of this same cobra. Temperature 70° Fah.

During the day it seemed to remain unaffected, nor did it appear affected during the subsequent fifteen days.

On the fifteenth day it was killed and dissected.

Post-mortem Examination.—Skin slightly dry and wrinkled over seat of injection, very faint localized staining subcutaneously at point where needle penetrated cuticle. The subjacent muscle here to extent of about size of split-pea semi-diffluent, but free from purulent products. Viscera strictly normal in appearance.

[1] One and a half grains dissolved in water killed in 20 minutes a chicken weighing 16 ozs.

To test the activity of the venom used in Experiment XX the following experiment was made immediately after injecting the cobra :—

EXPERIMENT XXI.—Two minims of the same solution as was used in Experiment XX were hypodermically injected into thigh of a fowl weighing 31 ozs.—

8-25 A.M.—Time of injection.
8-50 „ —Drooping in sitting position.
9-5 „ —Beak resting on ground.
9-15 „ —Lying on side.
9-50 „ —Dead.

Results of these experiments.

The results of these experiments unequivocally demonstrate that the cobra is practically, if not wholly, insusceptible to the toxic action of its own venom. In the first series, the cobras were kept under observation for nine days, in the second series for fifteen days, and in the third for fifteen days. And the fact that each series of experiments was conducted under different conditions as to locality,[1] elevation above sea-level, temperature, and season, tended to eliminate local sources of experimental error.

Review of Mitchell and Fayrer's anomalous experiments.

In view of the absolute uniformity of results which has obtained throughout these experiments, it becomes desirable to review the details of those experiments by Mitchell and Fayrer which seem to have been attended by exceptional or anomalous results, in order to ascertain whether the seemingly anomalous results may not be attributable to accident.

Fayrer's experiments on cobras *versus* cobras.

The experiments recorded by Fayrer upon cobra-venom *versus* the cobra are 23 in number, *viz.* 16 on cobras bitten by cobras and 7 in which cobra-venom was injected hypodermically into cobras. In these 23 experiments there were only three deaths. Reviewing the particulars of these three fatal cases it is to be remarked in regard to the *first* case,[2] that no *post-mortem* examination was made to ascertain whether the needle had not entered a blood-vessel or viscus, or the non-existence of any other obvious cause of death. Neither is it stated whether the snake was a fresh or vigorous one. In six further experiments of a similar nature the cobras are reported to have survived.

In regard to the *second* fatal case[3]—a young cobra "10 inches long" and "only 10 or 14 days old"—the marvel is that it survived so long (six days) in captivity after having been wounded by biting.

In the *third* case[4] the cobra was reported next day "perfectly well," and it seems to have lived for six days. The death may have been due to starvation,

[1] Two of the places were about 200 miles apart.
[2] *loc. cit.*, p. 75.
[3] *loc. cit.*, p. 99.
[4] *idem*, p. 120.

septicæmia, or other accidental causes. No *post-mortem* examination appears to have been made.

Thus, in regard to neither of these three cases is there evidence to show that the fatal event was due to the venom. On the contrary, the likelihood that death resulted from other and accidental causes is so great that these three experiments may well be eliminated from the series; and when so eliminated, there remain 20 experiments, with no deaths.

Mitchell's experiments on rattlesnakes *versus* rattlesnakes.

Mitchell records[1] seven experiments on rattlesnakes with rattlesnake venom, and concludes that "the above experiments were on the whole so definite in their results that I did not think it necessary to multiply them," and that these rattlesnakes "really died from the venom."

An examination, however, of the details of his experiments renders it difficult to conceive how Dr. Mitchell was led to such a conclusion.

But before examining the particulars of these experiments *seriatim*, it is necessary to remember, *firstly*, that these snakes had been for some time in captivity—the record of these experiments being prefaced by the remark "at the close of a series of experiments on warm-blooded (animals) I made use of some of my largest snakes in the following manner."[1] And, *secondly*, that these snakes appear to have been repeatedly subjected to extremely rough handling and forcible compression of the poison-glands during extraction of the venom—a process so severe that it is noted,[2] "One snake in every four died within two to five days" from the mere effects of the process for extracting their venom. And to this process the snakes of Experiments I, III, V, VI and VII, seem to have been subjected during their final experiment.

In Experiment I, the snake did not die till the fourteenth day after being bitten, and it presented no signs of viper-poisoning.[3] In Experiment II, the snake "died on the fourteenth day. The wound was apparently healthy.". . . "The blood was uncoagulated, but there was no other visible lesion of any internal organ." Concerning Experiment III, it is noted, "In all probability this serpent died from some other cause than venom-poisoning." In Experiment IV the snake "recovered," although it bit itself three times on a part from which skin had been removed, throwing out "a good deal of venom which was thrust deeply into the muscles of wounded part." "No blood was effused" and "at the close of two weeks this snake was healthy and bit eagerly." In Experiment V, although the snake lived for 36 hours after injection of poison, there appears to have been no extravasation of blood, except that the stomach "contained bloody mucus." The wound "was not stained with blood." "The heart was full of *clotted* blood." In Experiment VI, where the snake lived for about three days, it is recorded that the local appearances were even "less extensive" than in last

[1] *loc. cit.*, p. 61 *et seq.*

[2] *idem*, p. 29, the author remarking that "it is not impossible that too severe a compression of the venom-gland may produce rupture of its substance and consequent blood-poisoning."

[3] Fluidity of blood with bloody extravasations are the leading physical signs of viper-poisoning.

experiment. "The interior organs were healthy, and the heart contained loose soft clots." In Experiment VII, the snake died on the seventh day and had bloody extravasations into the peritoneal cavity, and the intestines were spotted with ecchymoses. But such appearances are common to death from septicæmia, and it is noted that the wound "penetrated the muscles, which were dark and much softened."

Mitchell's inference from his experiment scarcely warranted.

Thus, taking this series of Dr. Mitchell's observations as a whole, and keeping in view the above-noted sources of error to which these experiments were especially subject, we find that the results, far from "definitely" warranting the conclusion that the rattlesnakes "really died from the venom," can more readily be interpreted as supporting quite an opposite conclusion. And this opposite conclusion gains from Dr. Mitchell further support from his observation[1] that in the "numerous instances in which I had wounded the mouths of rattlesnakes or torn the *vagina dentis* while robbing them of poison, on none of these occasions have any serious results followed the injury, even where the venom had fallen upon the abraded surfaces in considerable amount."

General conclusion on the autotoxicity of venom.

Summarizing the results of all the foregoing observations, it will be seen that, when the suspicious elements are eliminated from the experiments of Mitchell[2] and of Fayrer, the results are in accordance with those of the present series of experiments, and also with the recorded rough experiments of Russell and of Richards upon cobras biting each other; and, that they generally confirm and extend the principle formulated by Fontana in regard to viper-venom, *viz.* "that the venom . . . is neither a poison to the snake itself nor to those of its own species."[3]

In inquiring into the cause of venomous snakes (all?) being immune to their own venom or that of their species, it becomes necessary to ascertain the effect of venom upon snakes of other species—venomous and non-venomous, as well as upon other cold-blooded animals. As to *warm-blooded* animals, all modern observers seem agreed in confirming the conclusion arrived at by Fontana from experiment on all the available warm-blooded animals in Italy, *viz.*, that venom "is a poison to *all* warm-blooded animals."[4]

Part III.—Effect of Venom on Venomous Snakes of other Species.

For experiment under this head, only pit-vipers, three in number, were available; my endeavours to procure other kinds of venomous snakes so late in the season having proved unsuccessful.

[1] *loc. cit.*, p. 63.
[2] Further experiments on rattlesnakes are desirable in order to remove all ambiguity.
[3] *loc. cit.*, p. 34.
[4] *loc. cit.* p. 273. Certain American writers consider the pig immune to venom (*New York Med. Jour.*, p. 54, 1884), but more authentic evidence on this point is needed.

Upon these three vipers the effect of cobra-venom was tried. The details of the experiments are as follow :—

Effect of cobra-venom on *Trimeresuri.*

EXPERIMENT NO. XXII.— A newly caught vigorous large *Trimeresurus erythrurus*, measuring 29½ inches in length, had, at 8-15 A.M., 4 grains of fresh dry cobra-venom of ascertained activity dissolved in 12 minims of water, and injected hypodermically about middle of back. Atmospheric temperature 82° Fah.—

8-15 A.M.—Time of injection.
8-18 „ —Actively crawling up sides of cage.
8-30 „ —Still active.
9 „ —Very quiet.
9-15 „ —Dead.

Post-mortem dissection.—Heart continued pulsating till 1-5 P.M. The injection had been strictly hypodermic. Muscles not wounded, and only a faint streak of capillary hæmorrhage at seat of injection. Viscera normal in appearance.

EXPERIMENT NO. XXIII.—A newly caught very active small *Trimeresurus erythrurus*, measuring 12¾ inches, had injected hypodermically about middle of back 8 minims of same solution of cobra-venom, of which 5 minims had killed a chicken weighing 1lb in 20 minutes. Atmospheric temperature 85° Fah.—

11-17 A.M.—Injection.
11-19 „ —Actively moving.
11-25 „ —Scarcely able to move.
11-29 „ —When turned over on back, unable to regain position.
11-36 „ —Exhibits no movement unless pinched, and then only faintly moves the tail and neck.
11-42 „ —Dead.

Post-mortem dissection showed the injection to have been purely hypodermic. The snake had been captured only a few minutes before, and was uninjured during capture, and during injection was not roughly handled.

EXPERIMENT NO. XXIV.—A freshly caught active *Trimeresurus gramineus*, measuring 27 inches long, had, at 8-45 A.M., 7 minims of fresh cobra-venom, diluted with an equal bulk of water, injected hypodermically about middle of back. Atmospheric temperature 82° Fah.—

8-45 A.M.—Injection.
8-50 „ —Actively moving.
9-15 „ —Scarcely able to move.
9-25 „ —Almost dead.
9-32 „ —Dead.

Post-mortem dissection showed that the injection had been very slightly intra-muscular as well as subcutaneous. Only faint streak capillary hæmorrhage locally. Viscera normal in appearance.

Effect of Cobra, *Daboia*, *Bungarus* and *Echis* biting each other.

I here present a summary of all the published observations I can find, relating to the toxic action of venomous snakes upon each other. The snakes comprise the Cobra, *Daboia*, *Bungarus fasciatus* et *cæruleus*, and *Echis carinata*. And the toxic effect was estimated by forcing one snake to bite another of a different species—

Nature of Observation.	Serial No. of Case.	Observer.	Page of 'Thanatophidin of India' where reported.	Time under observation after bite.	Result.	Remarks.
Cobra biting *Daboia russellii*.	1	Breton[1]	...	2 days .	"No effect."	
	2	Fayrer	73	5 „ .	„ „	
	3	„	80	6 „ .	„ „	
	4	„	85	Not stated .	"Remained well."	
	5	„	85	„ „ .	„ „	
	6	„	92	„ „ .	„ „	
	7	„	98	2½ days .	"No change."	
	8	„	101	7 „ .	„ „	
Cobra biting *Bungarus fasciatus*.	1	Fayrer	74	29 hours .	Died .	Had been twice bitten by a large cobra.
	2	„	85	4 days .	„ .	"Lung and thorax filled with blood," indicating visceral or vascular lesion.
	3	„	92	18½ hours .	„ .	Been bitten three times.
	4	„	100	5 days .	"Well and active."	Died thirteen days after bite.
	5	„	107	1 day .	Died .	"Appears to have received some injury about head and neck."
	6	„	108	2½ hours .	„ .	Bitten twice 'near tail.'
	7	„	119	5 days .	„ .	Day following bite "perfectly well." When died "wound had become putrid."
Cobra biting *Echis carinata*.	1	Fayrer	140	20 hours .	Died.	
	2	„	„	1 day .	„	
Cobra biting *Bungarus cæruleus*.	1	Fayrer	124	40 minutes	Died .	"The fangs of the cobra were heard to strike the *krait's* spine."
	2	Richards	125	1 day .	"Found dead."	It is noted, "Death cannot be attributed to rough handling."
	3	„	127	1 hour .	Died .	The *krait* (*Bungarus*) was only 1 foot 10 inches long, and the cobra was full grown.
	4	„	127	1½ days .	"Quite well"	*Krait* was 2½ feet long.
	5	„	142	9 „ .	"No result"	
Daboia russellii biting Cobra.	1	Russell[2]	...	Not stated .	"No symptoms."	
	2	Breton[1]	...	„ „ .	Not affected.	
	3	Fayrer	80	6 days .	"No effect."	
	4	„	83	1 day .	"No change."	
	5	„	„	44 hours .	Died .	The cobra was 'full grown.' No *post-mortem* examination seems to have been made.

[1] *loc. cit.*, p. 175.

[2] *loc. cit.*, p. 85.

Nature of Observation.	Serial No. of Case.	Observer.	Page of 'Thanatophidia of India' where reported.	Time under observation after bite.	Result.	Remarks.
Daboia russellii biting Cobra—*contd.*	6	Fayrer	83	1½ days .	Little, if any effect.	
	7	,,	,,	1½ ,, .	"No effect."	
	8	,,	85	2 ,, .	Little, if any effect.	
	9	,,	92	5 ,, .	"Quite well."	
	10	,,	99	4 ,, .	Died .	Cobra was only "10 or 14 days old."
	11	,,	101	7 ,, .	"No change."	
	12	Richards	127	1 day .	Died .	Death "possibly from injury done by the viper's enormous fangs." But no *post-mortem* examination seems to have been made.
Daboia biting *Bungarus fasciatus.*	1	Fayrer	100	13 days .	"Remained unaffected."	
Daboia biting *Bungarus cæruleus.*	1	Fayrer	140	Not stated .	"Unaffected"	
Bungarus fasciatus biting Cobra.	1	Fayrer	69	1 day .	"Well."	
Bungarus cæruleus biting Cobra.	1	Fayrer	120	2 days .	"Found dead."	Morning following bite was "unaffected."
	2	,,	133	7 ,, .	"Not, nor has been, affected."	
	3	,,	134	32 minutes.	Died .	Cobra was only 14 inches long, and *krait* was 48 inches.
	4	,,	140	4 days .	"Quite well."	
	5	Richards	125	3 ,, .	"Well." .	
	6	,,	127	1 day .	Died .	Cobra was of "small size."
	7	,,	142	2 days .	,,	It is noted, "The death may not be due to the bite, as many of the snakes are dying at present."
Bungarus cæruleus biting *Echis.*	1	Fayrer	138	Not stated .	"Not affected."	
Echis carinata biting Cobra.	1	Fayrer	136	1½ days .	Died .	During day of bite "no effect apparent."
Echis biting *Bungarus cæruleus.*	1	Fayrer	136	2 days .	"Quite well."	
	2	,,	138	Not stated .	"Not affected."	
	3	,,	140	4 days .	"No effect."	

Analytic summary of results.

In analyzing the experiments shown in the table, it is to be remembered that they were subject to most of the experimental errors already referred to in Part I, so that many of the experiments must be eliminated. When the series is thus modified many of the divergent results disappear; but much uncertainty must remain until more exact experiments are forthcoming. At present the nett result of these experiments may be stated as showing that—

(1) the Cobra-bite is inoperative on *Daboia russellii*, doubtfully operative on *Bungarus fasciatus* and *Bungarus cæruleus*, and fatal to *Echis carinata*;
(2) the *Daboia*-bite is inoperative on the Cobra and *Bungarus fasciatus* and *Bungarus cæruleus*; but the experiments with *Bungarus* being solitary ones and merely by biting are inconclusive;
(3) the *Bungarus cæruleus*-bite is inoperative on the Cobra and doubtfully so on *Echis*;
(4) the *Bungarus fasciatus*-bite is apparently inoperative on the Cobra;
(5) the *Echis*-bite is inoperative on *Bungarus cæruleus*.

My experiments showed that Cobra-venom is rapidly fatal to two species of the green tree-viper (*Trimeresurus*).

Part IV.—Effect of Venom on Innocent Snakes.

Own experiments with cobra-venom.

The results of my experiments upon the effect of venom on innocent snakes—16 in number—are shown in the following table. It is to be noted that all the snakes operated on had been newly caught, and, except where otherwise stated, vigorous and uninjured; the solution of venom was freshly made and subcutaneously injected about middle of back, and the activity of the venom solution had in every case been ascertained by control experiments on fowls. The snakes nearly always passed fæces during, or immediately after, the operation; but this was also done occasionally by the cobras while being gently manipulated and may be due to mere excitement. That the injection had been purely subcutaneous was ascertained by *post-mortem* dissection in every case, except where otherwise stated. The atmospheric temperature ranged between 80° and 86° Fah.

Experiments with Cobra-venom on Innocent Snakes.

Experiment No.	Species of Snake.	Length of Snake in inches.	Amount of Venom injected (approximately).	Result.	Duration of life from time of injection.	*Post-mortem* examination.	Remarks.
XXV .	*Ablabes rapii* . .	18½	½ grain	Died .	95 mins.	Very slight streak capillary hæmorrhage at seat of injection.	
XXVI .	*Simotes bicatenatus* .	26	¼ gr. and after 4 days 1½ grs.	Doubtful.	Doubtful.	.....	A very strong thick-set snake. Was still alive, although very sluggish second day after last injection, or sixth day after first injection.
XXVII .	*Tropidonotus subminiatus.*	22½	½ gr.	Died .	11 hours	No obvious exudation or discoloration at site of injection.	
XXVIII .	*Simotes bicatenatus* .	8¼	⅓ gr.	„ .	2¾ „	None made.	
XXIX .	*Dipsas gokool* . .	28½	1¼ grs.	„ .	3¾ „	„	
XXX .	*Dipsas gokool* . .	30¼	1¼ „	„ .	4 „	„	
XXXI .	*Simotes bicatenatus* .	24½	2 „	„ .	1¾ days.	Very slight inflammatory exudation, but no softening at site of injection. Viscera generally congested, intestines injected. No bloody fluid in cavities.	
XXXII .	*Dipsas hexagonata* .	32½	¾ gr.	„ .	5 hours .	None made.	
XXXIII .	*Dendrophis pictus* .	33½	¾ „	„ .	55 minutes	„	
XXXIV .	*Tropidonotus subminiatus.*	30½	1⅓ grs.	„ .	55 „	„	
XXXV .	*Tropidonotus quincunciatus.*	17⅝	½ gr.	„ .	10 hours.	At site of injection slight swelling from exudation, but no hæmorrhage.	
XXXVI .	*Uropeltis, sp.* . .	15	½ „	„ .	65 mins.	None made .	A young specimen.

Experiment No.	Species of Snake.	Length of Snake in inches.	Amount of Venom injected (approximately).	Result.	Duration of life from time of injection.	*Post-mortem* examination.	Remarks.
XXXVII.	*Gonyosoma gramineum*	$16\frac{1}{8}$	$\frac{1}{2}$ gr.	Died .	85 Mins.	None made .	Had received considerable flesh-wound during capture, but was newly caught and very active.
XXXVIII	*Ablabes collaris* . .	23	1 „	„ .	29 „	„	
XXXIX .	*Gonyosoma gramineum*	19	1 „	„ .	45 „	Injection proved to be partially intramuscular (very superficially) and attended by slight capillary hæmorrhage.	Had received severe wound below neck during capture.
XL .	*Tropidonotus quincunciatus.*	$37\frac{3}{4}$	2 grs.	„ .	$5\frac{3}{4}$ hours.		

Results of these experiments.

In all these experiments the cobra-venom proved to be more or less rapidly fatal.[1] The duration of life after injection of the venom was in direct ratio (*a*) to the amount of venom administered, and (*b*) to the robustness of the individual operated on: the more delicate tree-snakes rapidly succumbed, while the relatively shorter but more robust *Simotes* offered much greater resistance to the action of the venom. The snakes operated on were generally of small size, as the larger species of non-venomous snakes, *e.g.* the python and rat-snake (*Ptyas*), could not at the time be obtained for experiment.

Experiments of previous observers.

The results recorded by previous observers are shown in the following table. The snakes were *bitten* by the venomous species; the biting snakes, with two exceptions, being cobras. In addition to those given in the table are three experiments by Fontana[2] in which innocent snakes, called respectively 'aspick,' 'adder,' and 'orvai' were bitten by vipers; but beyond 'slight torpidity' in one case no other effect was noted.

[1] In experiment No. XXVI the fact of actual death was not ascertained; but this snake when last seen was evidently dying; and the deaths of two others of the same species were duly observed—*Vide* Experiments Nos. XXVIII and XXXI.

[2] *loc. cit.*, p. 39.

Experiments on Innocent Snakes bitten by Venomous Snakes.

No.	Biting Snake.	Bitten Snake.	Observer.	Length of Snake.	Result.	Duration of life from time of bite.	Remarks.
1	Cobra . .	'Nooni paragodoo' (*Zamenis fasciolatus* ?).	Russell[1] .	...	Died	1¼ hours.	
2	,, . .	'Tartutta' (*Dipsas trigonata*?).	,, . .	...	,,	2 ,,	
3	,, . .	'Dhonr' (*Tropidonotus sp.*?).	Breton[2] .	...	,,	2¼ ,,	
4	,, . .	*Ptyas mucosus* . .	Fayrer[3] .	...	*Nil.*	...	Only observed for one day.
5	,, . .	,, . .	,, .	8 feet .	Died	7 days .	3 days after bite reported "quite well."
6	,, . .	,, . .	,, .	6 ,, .	,,	1½ ,, .	Bitten at part where scales scraped off—the cobra had just fatally bitten a lizard.
7	,, . .	,, . .	,, .	...	*Nil.*	...	Only observed for one day.
8	,, . .	,, . .	,, .	...	,,	...	Only observed for one day.
9	,, . .	,, . .	,, .	'Full grown.'	,,	...	Only observed two days.
10	,, . .	,, . .	,, .	'Large'	,,	...	Had ten 'drops' cobra-venom (11 days old) injected hypodermically under mm. of mouth. 'Several days later' it was 'quite well.'
11	,, . .	*Tropidonotus quincunciatus.*	,, .	'Small'	Died	13 minutes.	
12	,, . .	*Dendrophis pictus* .	,, .	40 inches	,,	9 ,,	} Was not spine crushed
13	,, . .	,, . .	,, .	'Smaller' than above.	,,	7 ,,	} in biting?
14	,, . .	*Ptyas mucosus* . .	,, .	...	,,	6¾ hours .	Was bitten three times by two cobras.
15	,, . .	*Dendrophis (sp.)* . .	,, .	'Small'	,,	41 minutes	Bitten by two cobras.
16	,, . .	*Dryophis (sp.)* . .	,, .	3½ feet .	,,	36 ,,	,, ,,
17	,, . .	*Ptyas mucosus* . .	,, .	'Large'	,,	27½ hours.	
18	,, . .	,, . .	,, .	'Small'	,,	21 minutes	Twice bitten.
19	,, . .	*Passerita mycterizans* .	,, .	3 feet .	,,	2 ,,	"Body swollen where bitten." (*Qu.* from hæmorrhage?) No *post mortem* exam. made.
20	,, . .	,, ,, .	,, .	'Small'	,,	17 ,,	
1	Daboia . .	*Ptyas mucosus* . .	,, .	'Large'	Died	*	* After bite 'sluggish.' "Recovered subsequently." On seventh day again bitten and died in 1½ days.
1	*Bungarus cæruleus.*	*Ptyas mucosus* . .	,, .	2 feet .	Died	5½ hours .	

[1] *loc. cit.*, p. 56.
[2] *Idem*, p. 170.
[3] *Idem*, p. 66 *et seq.*

General summary of results.

In these experiments the smaller snakes all died more or less rapidly from the cobra bite, in accordance with the results of my own experiments with cobra-venom as previously given. In the case of the large and powerful 'rat-snake' (*Ptyas mucosus*), however, the results were very varied. This want of uniformity is doubtless in part owing to the experimental errors incidental to biting as a means of introducing the poison, and to insufficiently long observation. But, after allowing for these sources of error, the series of experiments seems sufficient to indicate that the full-grown rat-snake offers a considerable resistance to the action of the venom.

Experiments on pythons, and further and more exact experiments on rat-snakes, are desirable.

Part V.—Effect of Venom on other Cold-blooded Animals—vertebrate and invertebrate.

A considerable number of observations are available under this head.

On Alligator.

Mitchell refers[1] to an experiment with rattlesnake venom upon an alligator as "well illustrating . . . . the activity with which venom may be absorbed by mucous membranes."

On Lizards.

Fontana[2] found that the smaller lizards "scarcely survive its (the viper's) bite for a few minutes." Fayrer[3] records two cases of the large water-lizard (*Varanus flavescens*) bitten by cobras with a fatal result in 27 and 46 hours respectively. Wall reports[4] a lizard (*Calotes versicolor*) dying in 10 minutes from 4cc. of *daboia* venom.

On Tortoise and Turtle.

Fontana[5] recorded 11 experiments on turtles with the relatively mild viper-venom: three of the turtles died and eight were practically unaffected. Cantor[6] relates two cases of Tortoise (*Trionyx gangeticus*) bitten in the lip by sea snakes: the one bitten by a *Hydrophis schistosa*, 2 feet 9 inches in length, died in 28 minutes; and the other bitten by a *H. striata*, 3 feet long, died in 46 minutes.

On Frogs.

Fontana records[7] results of experiments on 86 frogs bitten by vipers, and found that "some died in less than half an hour, others in an hour, and others again in two or three hours;" And that generally frogs "die in a few hours if bitten by a viper." Mitchell records[8] experiments on ten frogs with *crotalus*-venom and all died:

[1] *loc. cit.*, p. 46.
[2] *loc. cit.*, p. 41.
[3] *loc. cit.*, pp. 68 and 70.
[4] '*Indian Snake poisons.*' London, 1883, p. 62.
[5] *loc. cit.*, p. 39.
[6] *Trans. Zool. Soc.* (Lond.) Vol. II, 1841, p. 310.
[7] *loc. cit.*, p. 97 *et seq.*
[8] *loc. cit.*, p. 55.

two dying in an hour, and two living for three to five days. Fayrer gives an account[1] of three large frogs (*Rana tigrina*) bitten by cobras: two of these died in 26 minutes and in 60 minutes respectively, while the third, which had been bitten by a cobra which had just bitten other animals four times, was reported next day to have "remained quite well."

Brunton and Fayrer[2] give three experiments on frogs with small doses of cobra-poison—the frogs dying within 12 hours. Several other frogs were poisoned by them with cobra-venom to observe the effects on nervous and muscular irritability. Wall reports[3] four frogs injected with cobra-venom dying in 40, 67, 79 minutes, and 4 hours respectively; and one frog as recovering from ·77 grs. *daboia*-venom, while a similar amount of cobra-venom proved fatal in about an hour.

Own experiments on Frogs.

Of my experiments on frogs, the leading results are here tabulated. The venom was in watery solution and injected into the dorsal lymphatic sac. Atmospheric temperature ranged from 80° to 86° Fah. Control experiments were made on two frogs similar in size to the first two of the series by injecting a similar quantity of water into sac; but the frogs remained unaffected, and on the second day they were still well.

Experiments with Cobra-venom on Frogs.

Experiment No.	Species of Frog.	Size.	Amount of Venom injected (approximately).	Result.	Duration of life from time of injection.	*Post-mortem* examination.	Remarks.
XLI.	Ground frog[4] .	Medium	$\frac{1}{3}$ grain .	Died.	15 minutes	Appearances normal.	
XLII.	„ .	„ .	$\frac{1}{3}$ „ .	„	13 „ .	. Ditto.	
XLIII.	*Bufo galeatus* .	Large .	1 gr. dried venom.	„	$27\frac{3}{4}$ hours	Wound slightly swollen and congested.	One grain freshly dried (without heat) venom was applied to wounded crural muscle through valvular cutaneous opening.
XLIV.	*B. melanostictus*	Very large.	$1\frac{1}{2}$ grs. dried venom.	„	48 „ .	Wound softened and sloughing.	Applied as above.
XLV.	Ground frog .	Largish	$\frac{1}{2}$ grain .	„	32 minutes	Appearances normal.	
XLVI.	„ .	Medium	$\frac{5}{8}$ „ .	„	9 „ .	Ditto.	
XLVII.	*Polypedatus maculatus* . .	Large .	$\frac{3}{4}$ „ .	„	28 „ .	.....	
XLVIII.	Ground frog .	Medium	1 gr. dried venom.	„	85 „ .	.....	Applied as above.
XLIX.	*Polypedatus maculatus* . .	Large .	$\frac{3}{4}$ grain .	„	25 „ .	.....	
L.	*Bufo sp.* . .	„ .	$1\frac{1}{3}$ grains .	„	About 11 hours.	... ..	
LI.	„ . .	„ .	$1\frac{1}{2}$ „ .	„	5 hours .	.....	

[1] *loc. cit.*, pp. 68 and 70.

[2] *Proc. Roy. Soc.*, Vol. XXII (1874), p. 73.

[3] *loc. cit.*, p. 10 *et seq.*

[4] All the ground frogs used were of the same species—which I have not yet identified.

On Fish.

Fontana found[1] that from the viper-bite eels "die later" than "the other kinds of fish" he had experimented upon, and not until the end of 18 or 24 hours. Fayrer reports[2] a case of a fish (*Ophiocephalus marulius*), 14 inches long, bitten near the tail by a cobra dying in 50 minutes.

On Invertebrates and Plants.

Upon leeches[3] spermatozoa and infusoria, on many of the lower forms of plants[4] and on cell movement in *Vallisneria*, &c.,[5] snake-venoms have been found to have little or no toxic effect.

Part VI.—What is the Cause of the Serpents' Immunity from their Venom?

How is the organism of the Venomous Snake able to resist the action of a chemical poison[6] which is fatal to most other animals?

Having obtained information concerning the effect of venom upon several other species of snakes—venomous and non-venomous—and on various other animals, we are now in a better position for considering the difficult problem here presented.

Fact of being cold-blooded.

The immunity is not to be explained upon the mere fact of the animal being cold-blooded. For, although venom acts *cæteris paribus* with greater force and rapidity on warm-blooded animals, and most so on those whose temperature is highest, *viz.* birds; yet, as we have seen, the other reptiles and other cold-blooded animals were affected by venom, and in several instances with a severity and rapidity little inferior to that exhibited by warm-blooded animals.

Fact of possessing anatomical form peculiar to ophidians.

Nor can the fact of having the anatomical conformation peculiar to ophidians explain the immunity. For, as we have seen, most, if not all, the non-venomous snakes are susceptible to venom. As Fontana long ago remarked (7)" if a comparison be instituted between two cold-blooded animals—one that dies of the disease of the venom, and the other that survives its action—they will be found to possess the same organs, the same circulation, an equal tenaciousness of life, and they will both of them appear perfectly alike . . . . what is it then that causes the venom to be a poison to the one, and not to the other?

[1] *loc. cit*, p. 41. [2] *loc cit.*, p. 68. [3] Fontana *loc. cit.*, p. 35.

[4] Mitchell, *loc. cit.*, p. 52. *Vide* also foot-note, p. 25, Part VI.

[5] Brunton and Fayrer, *loc. cit.*

[6] Snake-venom possesses all the characters of a strictly *chemical* poison as opposed to those of a *living* ferment or enzyme. Amongst the direct evidence on this head are the experiments by Albertoni and Bufalini with the blood of envenomed animals (*Rivista di chimica Med., Farm.*, December 1883). And culture-experiments by Prof. Formad of Philadelphia in regard to *crotalus* venom (Mitchell and Reichert "*Researches upon the Venoms of Poisonous Serpents*" [Smithson. Contrib.], Washington, 1885, p. 137) and by Dr. Wolfenden in regard to cobra venom (*Ind. Med. Gaz.* 1885), gave negative results.

[7] *loc. cit.*, p. 276.

Fact of possessing a poison-apparatus.

Neither does the mere possession of an active poison-apparatus secure immunity. For, as we have seen, cobra-venom proved rapidly fatal to the tree-vipers experimented upon.[1] And the *Dipsadæ*, which possess grooved fangs, and thus presumably related to the venomous snakes, were found[2] to be much more susceptible to venom than the relatively much smaller and wholly innocent *Simotes*.[3]

In what manner, then, is the immunity to be explained?

Hypothesis offered in explanation of immunity.

In answer to this I have at present only an hypothesis to offer: From what has gone before, it would appear that the venomous snake does not secure its immunity through any peculiarity in general structure or physiology *per se*; nor, does the mere possession of an active-poison apparatus render the snake immune to the venom of a more highly virulent species. The one striking feature, which is uniformly associated with the immunity, is the possession of a venom of a degree of virulence and other qualities special to the particular species;[4] and we have seen that snakes are habitually swallowing some of their own venom, and absorbing small quantities through wounds and abrasions within the buccal cavity. Under these circumstances, is it not possible that the immunity may be an acquired condition—a toleration to the venom established through frequent imbibition of small quantities of the venom in the modified or attenuated form which the venom assumes when mixed with salivary and gastric juices, and absorbed through the alimentary canal?[5]

[1] Implying that there are degrees of immunity.

[2] *Vide* Experiments 29, 30, and 32.

[3] *Vide* Experiments 26, 28, and 31.

[4] Regarding the probable inter-immunity of the Cobra and *Daboia*, *vide* pp. 63 and 72.

[5] Mitchell and Reichert, *loc. cit.*, p. 44, reviewing the published observations upon the absorption of venom by mucous surfaces, state that "the verdict of all observers in connection with the venom of the crotalidæ is that uninjured mucous surfaces, except in the lungs, cannot absorb venom, at least in sufficient quantities to produce death. In experiments with the venom of the cobra other investigators have gotten results which are directly contrary; but in our own researches (details of which are given) a large proportion of the animals survived." And experiments are given to show that "the gastric juices . . . are destructive of venoms."

On the *digestion* of cobra-venom I have the two following experiments:—

Experiment LII.—Of a solution of cobra-venom, of which 8 minims killed a chicken weighing 14 ounces in 14 minutes, 15 minims were taken, and to this were added 15 minims of a freshly prepared and faintly acid solution of *Pepsine* (10 grs. to oz. of water) of ascertained activity; and the mixture was digested for 20 hours at a temperature ranging between 83° to 90° Fah. The solution was then filtered, and the whole of the filtrate injected hypodermically into thigh of a chicken weighing 16 ozs. The chicken died in 70 minutes with all the symptoms of cobra-poisoning.

Experiment LIII.—Of the same solution of cobra-venom as was used in above experiment 10 minims were added to 20 minims of a freshly-prepared and faintly acid solution of *Papaine* (10 grs. to oz. of water) of ascertained activity; and the mixture digested for 21 hours at a temperature ranging between 83° to 90° Fah. The solution was then filtered, and the whole of the filtrate injected hypodermically into thigh of a chicken weighing 14 ozs. The chicken died in 58 minutes with symptoms of cobra-poisoning.

These experiments would tend to show that digestion modifies the virulence of cobra-venom: thus, of a solution of venom of which 8 minims killed a chicken weighing 14 ozs. in 14 minutes, 15 minims (a slight loss would occur through filtration) when digested with pepsine failed to kill a chicken weighing 16 ozs. until 70 minutes; and 10 minims when digested with papaine failed to kill a chicken weighing 14 ozs. until 58 minutes.

In support of this hypothesis the following additional arguments may be adduced:

Additional arguments favouring hypothesis.

Injection of *purely chemical* material may, it is reported, procure immunity from a specific disease: the protection conferred by 'vaccine' being, it is alleged, attributable, in certain cases, to the action of the soluble chemical products resulting from the growth and development of the morbific germ, rather than to any direct action of the fungus itself.[1]

Injection of *purely chemical* material alleged to procure immunity from specific disease.

Venom is not a general protoplasm-poison,[2] so that its presence need not necessarily be incompatible with cell life.

Venom not a general protoplasm-poison.

It is an established fact that cells may, by a process of education, become habituated to nutriment, which they at first reject when it is presented to them in a concentrated form.[3] It is thus not impossible that the protoplasm of the snake may become gradually habituated to the presence of this extraneous proteid material-venom.

Habituation of protoplasm to foreign food.

It has long been suspected that certain snake-charmers by a process of inoculation with venom gain protection against the bite of a particular species of venomous snake.[4] And this belief was not effectually disposed of by Fayrer's experiments on the hurriedly prepared dogs submitted by him to snake-bite.[5]

Alleged practice of inoculation among snake-charmers.

[1] M. Bouchard and others have demonstrated (says the *Lancet*, 1888), that a rabbit may be protected against the pyocyanic disease by injecting under its skin the soluble products of cultures deprived of all microbes by heat and filtration; and that immunity can also be secured by injecting sterilized urine of rabbits dead from pyocyanic infection, and MM. Charrin and Ruffer (*France Med.* No. 126) find that the soluble products of artificial cultures are eliminated by the kidney without losing their power of conferring immunity. Dr. Wooldridge ("Note on Protection in Anthrax," *Proc. Roy. Soc.*, 1887, p. 313), found as regards anthrax that the albuminous culture-fluid freed from anthrax bacilli (and spores?) by filtration confers protection. Confirmation, however, of such experiments by Koch or other thoroughly reliable observer is needed. As it is highly remarkable that strictly soluble material can make so very lasting an impression on the system.

[2] The evidence on this point is extensive, *viz.*—Fontana's experiments with viper-venom on leeches *l. c.* p. 36. Mitchell's experiments with *Crotalus*-venom on rotifers "and other forms of animalcular life, and on fungi, *loc. cit.* pp. 50—52. Darwin's experiment with cobra and adder-venom on *Drosera*,—'*Insectivorous Plants*' Lond., 1875, p. 206 *et seq.* Brunton and Fayrer's experiments with cobra-venom on leucocytes, ciliary motion, cell-movement in vallisneria, and on germinating seeds, *Proc. Roy. Soc.*, 1875., p. 278, Vol. 23. Mitchell and Reichert's experiments with rattlesnake-venom on ciliary movement, spermatozoa, &c., *loc. cit.*, p. 149 *et seq.* A. E. Feoktistow's experiments with venoms of *Vipera ammodytes*, *v. berus*, and *crotalus durissus* on spermatozoa, monads, and bacteria.—*Trans. Imp. Ac. Sci.* St. Petersburg, 1888, quoted in *Lancet*, p. 333, Vol. II, 1888.

[3] Thiselton-Dyer in *Nature*, p. 480, 1888.

[4] A popular belief, shared by Nicholson, but apparently a mere conjecture (*loc. cit.*, p. 148); and by, if I rightly remember, Dr. Stradling, but at present I am unable to refer to the published views of the latter.

[5] *Thanatophidia of India.*

This hypothesis would also lend itself to explain the relative or absolute protection possessed by one species of highly virulent snake against another highly venomous snake of a different genus or family (*e.g.* the *Daboia* against the Cobra). For, Mitchell and Reichert have shown[1] that the various serpent-venoms examined by them are mixtures of two or three proteids (*viz.* 'Venom-albumin,' 'V.-globulin,' and 'V.-peptone'); and that the venom of one species of snake differs from that of another, mainly, if not entirely, in the different proportions in which these constituent proteids are present. The *Daboia* and Cobra might, in this way, be regarded as being protected against each other[2] to that extent to which their venom contains a corresponding proteid, and thus have to contend against only a moiety of the poison.[3]

The inter-immunity of venomous snakes explainable.

The hypothesis here put forward can readily be tested by experiment; and to this test I propose submitting it when opportunity offers.

[1] *loc. cit.* And Dr. WOLFENDEN has generally confirmed this observation as regards cobra-venom (*Ind. Med. Gaz.*, 1885).

[2] *Vide* p. 63.

[3] The venoms of the Cobra and *Daboia* appear to have much in common: "The general symptoms produced by the poison of the *Daboia* are nearly the same as by that of the *Naja*." BRUNTON AND FAYRER, *loc. cit.*, p. 68.

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